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Endocannabinoid turnover

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Contents

References

1. Introduction

In this report, we assess the principle routes by which the endocannabinoids anandamide and 2-arachidonoylglycerol are produced in biological systems, and their hydrolysis and biotransformation.

Anandamide (AEA, N-arachidonoylethanolamine) and 2-arachidonoyl-sn-glycerol (2-AG) are arachidonic acid derivatives which are synthesised and hydrolysed by independent, parallel pathways (Figure 1). However, a feature they share with arachidonic acid is the possibility of biotransformation, via oxidative metabolism (Figure 1, see below). We review the enzymes responsible for endocannabinoid turnover, as well as the potential for therapeutic exploitation.

2. Biosynthesis of AEA and related NAEs

The synthesis of AEA and NAEs has been well reviewed elsewhere (Ueda *et al.*, 2013) and so will be dealt with briefly here. The pioneering work on NAE synthesis was undertaken by Harald and Patricia Schmidt and colleagues who delineated the key steps. The first step is the transacylation of membrane phosphatidylethanolamine-containing phospholipids to form *N*-acyl-phosphatidylethanolamines (NAPEs) by a calcium-dependent *N*-acyl transferase (Natarajan *et al.*, 1982; Natarajan *et al.*, 1983) that has recently been identified as the cytosolic enzyme PLA2G4E (Ogura *et al.*, 2016). Calcium-independent formation of NAPEs from phospholipids are catalysed by a family of phospholipase A/acyltransferase (PLA/AT) enzymes, where PLA/AT 1, 2 and 5 have greater *N*-acyltransferase activity than phospholipase 1/2 activity (Uyama *et al.*, 2012). NAPEs are thereafter metabolised to the corresponding NAEs by a variety of pathways, including hydrolysis catalysed by NAPE-hydrolysing phospholipase D (Schmid *et al.*, 1983); production and subsequent dephosphorylation of NAE phosphates (Liu *et al.*, 2006; Okamoto *et al.*, 2004a), and production and hydrolysis of lyso-NAPE (Sun *et al.*, 2004). Finally, NAEs can be produced from *N*-acylethanolamine plasmalogen (by both NAPE-PLD dependent and independent pathways (Rahman *et al.*, 2016; Tsuboi *et al.*, 2011). Consistent with this multiplicity of pathways, genetic deletion of NAPE-PLD reduces, but does not remove completely, NAE levels in the brain (Leishman *et al.*, 2016; Leung *et al.*, 2006;

Tsuboi *et al.*, 2011). The situation is further complicated by the finding that NAPE-PLD can be allosterically modulated by bile acids (Margheritis *et al.*, 2016; Schmid *et al.*, 1983).

Given the central position of NAPE as a precursor to NAE, it would be expected that the levels of the NAE species match those of NAPE and of the membrane phospholipids. In the brain, this is the case. As an example, data from Hansen and colleagues (Hansen *et al.*, 2001a; Hansen *et al.*, 2001b) investigating levels of these lipids in young rat cerebral cortex are shown in Figure 2. There is a very good correlation between the levels of the NAPE and corresponding NAE species, with a large increase in both following intrastriatal injection of the glutamatergic excitotoxin *N*-methyl-D-aspartate (NMDA). In all cases, the NAE levels are ~10% of the corresponding NAPE levels. However, there are no rules without exception (a rule in itself!) and the exception in this case is the uterus, where endocannabinoids play a key role in reproductive behaviour (review, see (Paria *et al.*, 2002)). In this case, the NAEs other than AEA are again roughly 10% of the corresponding NAPE levels, whereas AEA levels are much higher than expected (Figure 3).

A second observation relates to the calcium sensitivity of PLA2G4E (Ogura *et al.*, 2016), and of NAPE-PLD (Okamoto *et al.*, 2004b). Such calcium sensitivity presents an obvious regulation point, whereby extracellular events leading to an increased intracellular calcium will stimulate NAE synthesis. Indeed, NAPE synthesis in cultured cortical neurons is increased following treatment with the calcium ionophore ionomycin in a manner potentiated by treatments increasing cyclic AMP levels in the cells (forskolin, vasoactive intestinal peptide) (Cadas *et al.*, 1996). In vivo, toxic events leading to calcium influx, such as NMDA treatment (Hansen *et al.*, 2001a; Hansen *et al.*, 2001b) [see Figure 2], traumatic injury (Garcia-Ovejero *et al.*, 2009) and oxygen deprivation (Amantea *et al.*, 2007; Franklin *et al.*, 2003; Schmid *et al.*, 1995) lead to increased NAPE-PLD activity and NAE levels. However, in murine peritoneal macrophages, treatment with the calcium ionophore A23187 does not increase the levels of either NAEs or NAPEs (Kuwae *et al.*, 1999). In macrophage cell lines, AEA (as well as *N*-acyltransferase and NAPE-PLD activities) but not 2-AG levels can be increased by lipolysaccharide

treatment, whereas the reverse is true when platelet-activating factor is used as a stimulus (Berdyshev *et al.*, 2001; Liu *et al.*, 2003). This is compounded by different expression levels of the AEA and 2-AG synthetic enzymes in different cells. A nice example of this comes from the work of Freund, Katona and colleagues who demonstrated that NAPE-PLD is located pre-synaptically in hippocampal glutamatergic axon terminals (Nyilas *et al.*, 2008)), in contrast to the synthetic machinery for 2-AG. Clearly, AEA/NAE and 2-AG syntheses are regulated differentially in a cell- and stimulus-dependent manner.

3. DAGL-dependent synthesis of 2-AG

2-AG was the second putative endocannabinoid to be identified a few years after AEA, acting as a full agonist at CB_1 and CB_2 receptors (Mechoulam *et al.*, 1995; Sugiura *et al.*, 1995). Interestingly, prior to this 2-AG was largely studied as an intermediate in a two-step pathway that releases arachidonic acid (AA) from diacylglycerol (DAG) in platelets and other cells. For example, thrombin treatment of human platelets was postulated to stimulate the generation of diacylglycerol (DAG) by a phospholipase C (PLC), followed by the sequential hydrolysis of DAG by a diacylglycerol lipase (DAGL) acting at the sn-1 position to generate 2-AG, and a monoacylglycerol lipase (MAGL) acting at the sn-2 position to liberate AA (Bell *et al.*, 1979; Prescott and Majerus, 1983). Subsequently other groups went on to confirm the release of AA through a DAGL/MAGL pathway in platelet-derived growth factor-stimulated Swiss 3T3 cells (Hasegawa-Sasaki, 1985) and in dorsal root ganglion neurons (Allen *et al.*, 1992). Remarkably, the above pathway for AA synthesis is conspicuous by its absence from some sections of the mainstream literature based on the continuing dogma that AA levels are largely governed by phospholipase A_2 (PLA_2) activity (Bazinet and Laye, 2014). We will discuss recent experiments that have provided unequivocal evidence for DAGL regulating the level of AA in the nervous system and some other tissues, and confirm the importance of DAGL activity for 2-AG biosynthesis and eCB signalling throughout the nervous system.

The first reported biochemical purification of a DAGL was in 1984 (Farooqui *et al.*, 1984); the authors identified a ~27 kDa enzyme that was not directly activated by calcium, but subsequently reported to be phosphorylated and activated by cAMP-dependent protein kinase (Rosenberger *et al.*, 2007). However, it was a bioinformatics strategy that resulted in the cloning and characterisation of the first specific DAGLs (Bisogno *et al.*, 2003). A single enzyme is present in lower species such as *Drosophila* and nematodes, but a gene duplication event has resulted in the presence of two very closely related enzymes, namely DAGLα and DAGLβ, in vertebrates and other species. These enzymes are not obviously related to the above 27 kDa DAGL, as both have an N-terminal four transmembrane domains followed an intracellular canonical α/β hydrolase domain resulting in a molecular mass of 70-125 kDa. The presence of a C-terminal tail (~300 amino acids) in DAGLα is the main distinguishing feature between itself and DAGLβ. The transmembrane domain and the C-terminal tail are not required for enzyme activity (Singh *et al.*, 2016) and most likely regulate trafficking and compartmentalisation of the enzymes within polarised cells (Reisenberg *et al.*, 2012). Functional evidence suggests differential expression of the two enzymes in the CNS, with DAGLα being present in isolated neurones and DAGLβ expressed in microglia (Viader *et al.*, 2016). The catalytic activity of both enzymes has been characterised in detail; they are *sn*-1 specific DAGLs that can make and release 2-AG from cells in response to calcium stimulation (Bisogno *et al.*, 2003). Interestingly, although the enzymes selectively act at the *sn*-1 position, they show little preference for substrates with differing lipid groups at the *sn*-2 position which can include arachidonic, oleic, linoleic and stearic acid suggesting that they can synthesise a range of monoacylglycerols. This makes sense of their expression in species such as *Drosophila* that do not synthesise AA; here the enzymes are more likely to synthesise lipids such as 2-linoleoyl glycerol (Tortoriello *et al.*, 2013).

4. 2-AG, AA and AEA levels in DAGL knockout mice.

The contribution that the cloned DAGLs make to the biosynthesis of 2-AG, and the requirement of this for eCB signalling, has been directly addressed by the generation of independent lines of

knockout mice. Given the importance of eCB function for behaviour, we largely focus our attention on eCB levels in the adult brain and on the requirement of the cloned DAGLs for synaptic function. Other DAGL-dependent functions, including roles in axonal growth and guidance in the developing brain and the regulation of neurogenesis in the postnatal brain have recently been reviewed elsewhere (Maccarrone *et al.*, 2014; Oudin *et al.*, 2011).

Expression will dictate function and in the first cloning paper DAGLα transcripts were reported to be enriched in the nervous system with protein expression seen at relatively high levels in neuronal dendrites that appose CB_1 positive synaptic terminals (Bisogno *et al.*, 2003). However, DAGLα and DAGLβ are present in neural stem cells in the adult brain (Goncalves *et al.*, 2008) and more recent RNA-sequencing experiments on isolated cell types from the mouse cerebral cortex clearly show DAGLα transcripts to be present in astrocytes, neurons, oligodendrocyte precursor cells and microglia (Zhang *et al.*, 2014). In contrast, the same database shows that DAGLβ transcripts are found almost exclusively in microglia. Thus DAGLα is well placed to regulate the 2-AG levels in several cell types in the brain, with DAGLβ perhaps contributing alongside DAGLα in a more restricted manner within neural stem cells and microglia, the "resident macrophages" within the brain.

At the level of the whole brain and spinal cord 75-80% reductions in 2-AG levels have been observed in DAGLα knockout mice, with ~50% reductions seen in adipose tissue and liver (Gao *et al.*, 2010). Similar reductions of ~80% (or more) have been reported in cerebellum, hippocampus, striatum, whole cortex, prefrontal cortex and amygdala in three additional DAGLα knockout lines (Jenniches *et al.*, 2016; Shonesy *et al.*, 2014; Tanimura *et al.*, 2010), with a complete loss of stimulus generated 2-AG (Tanimura *et al.*, 2010) and no difference between the sexes (Shonesy *et al.*, 2014). The one anomaly appears to be the forebrain where significant, but less dramatic decreases have been reported (Shonesy *et al.*, 2014). Overall, these data indicate that DAGLα is responsible for

maintaining nearly all of the basal 2-AG in the nervous system, as well as being required for stimulus induced increases.

Nonetheless, residual 2-AG is found in the nervous system of DAGLα knockout mice implicating DAGLβ and/or other enzymes in the biosynthesis of this lipid. 2-AG levels were variable but not significantly different in the cerebellum, hippocampus and striatum of one line of DAGLβ knockout mice (Tanimura *et al.*, 2010). However, a 50% decrease in 2-AG at the level of the whole brain has been reported in an independent DAGLβ knockout line (Gao *et al.*, 2010). Thus, it would appear that DAGLα is primarily responsible for the biosynthesis of 2-AG in the brain, but DAGLβ can make a contribution, possibly within a restricted set of cell types (see above for details).

As discussed above, DAGL activity was first studied in the context of a DAGL/MAGL pathway that generates AA in cells yet much of the mainstream literature remains focussed on the notion that the bulk and signalling pools of AA are governed by the activities of the PLA_2 family of enzymes (Bazinet and Laye, 2014). In this context, it was a major surprise to see parallel reductions in AA and 2-AG levels in the brains of DAGLα knockout mice. For example, in the brain and spinal cord there are 75-80% reductions in AA in DAGLα knockout mice (Gao *et al.*, 2010). Similar dramatic reductions in AA are seen in the forebrain, prefrontal cortex, amygdala and striatum in independent lines of DAGLα knockout animals (Jenniches *et al.*, 2016; Shonesy *et al.*, 2014; Tanimura *et al.*, 2010). It follows that the DAGLα/MAGL pathway is responsible for the biosynthesis of most of the 2-AG and AA throughout the brain. Importantly, this pathway also appears to provide the signalling pool of AA that is mobilised to generate eicosanoids in the brain, with up to 80% reductions in the inflammatory prostaglandins PGD_2 and PGE_2 seen in DAGLα knockout mice (Ogasawara *et al.*, 2016). Inhibition of the eicosanoid pathway at the level of MAGL dramatically reduces much of the neurodegeneration seen in animal models of Alzheimer's and Parkinson's disease (Nomura *et al.*, 2011; Piro *et al.*, 2012), with inhibition at the level of DAGL impairing lipopolysaccharide-induced anapyrexia (Ogasawara *et al.*, 2016). Thus it is clear that the DAGL/MAGL pathway can generate prostaglandins that promote

neuroinflammation in the brain. Interestingly, in the periphery evidence from knockout and pharmacological studies also show that DAGLβ regulates proinflammatory responses by regulating 2-AG, AA and eicosanoid levels in a manner that is distinct but complementary with cytosolic PLA_2 (Hsu *et al.*, 2012).

A perhaps even more surprising observation made in the DAGLα knockout mice studies was a reduction in the level of AEA in the brain. In this context, there is a significant 40-50% reduction of AEA in the whole brain of DAGLα knockout mice (Gao *et al.*, 2010; Ogasawara *et al.*, 2016). The reason for this unexpected cross-talk between the biosynthesis of the two major eCBs does not appear to involve an obviously straightforward biosynthetic pathway (Di Marzo, 2011), but it is clearly a robust finding and on that basis it can be concluded that DAGLα is the key enzyme in a simple pathway that generates the bulk of the 2-AG and AA found in the brain, but it can also contribute in a more complex manner to the steady-state levels of AEA levels in some brain regions. We do not as yet know the consequence of knocking out DAGLα and DAGLβ on 2-AG levels in the brain, and it remains possible that a small pool of 2-AG (perhaps 10-20% of the total) might be maintained by a different biosynthetic pathway. In this context, an enzyme previously characterised as a phospholipase A_1 (and termed DDHD2) has recently been purified from the brain and cloned based on exhibiting DAGL activity with evidence obtained that it can contribute to bulk 2-AG levels following overexpression in CHO cells (Araki *et al.*, 2016). The importance of this enzyme in regulating the bulk levels or stimulus induced signalling pools of 2-AG in cells and tissues remains to be determined.

5. Pharmacological inhibition of the DAGLs mimics the knockout results

Genetic approaches to study enzyme function need to be interpreted with some caution given that the relatively long-term loss of function in one pathway can lead to alterations in other pathways with these secondary changes influencing the measured endpoints. On the other hand, whereas pharmacological approaches have the advantage of temporal resolution, they can be limited by drug

availability and selectivity; for review, see Janssen and van der Stelt (2016). In the case of the DAGLs, the historical pharmacological studies have largely relied on the use of RHC80267 and/or tetrahydrolipstatin (THL), but results obtained with them have sometimes been contentious as although they can inhibit DAGL activity they also inhibit other serine lipases and can show poor tissue penetration (Hoover *et al.*, 2008; Janssen *et al.*, 2015). A set of centrally active DAGL inhibitors has recently been developed that, when used with appropriate controls, has allowed for the analysis of the effects of acute inhibition of DAGL activity on lipid networks in the brain (Ogasawara *et al.*, 2016). These 1,2,3-triazole urea-based drugs are highly selective across the discernible family of ~~alpha~~α/~~beta~~ β domain serine hydrolases, but show limited selectivity between DAGLα and DAGLβ. Within 2 h~~r~~ administration to animals, these novel inhibitors (DH376 and DO34) fully mimic the reductions in 2-AG (>80%), AA (>80%) and AEA (~50%) measured in parallel studies on DAGLα knockout mice. Concomitant increases in the level of the 2-AG precursor 1-stearoyl-2-arachidonyl-*sn*-glycerol were also seen in the drug treated and knockout animals. Importantly, the levels of the prostaglandins PGD_2 and PGE_2 were also rapidly reduced by ~80% following pharmacological block of DAGL activity, as were the LPS induced increases in these prostaglandins and inflammatory cytokines demonstrating the importance of the DAGLα/MAGL pathway for the rapid generation of inflammatory mediators in the brain. LEI105 represents a distinct class of reversible DAGL-selective inhibitors, based on α-ketoheterocycles (Baggelaar *et al.*, 2015). The use of this compound in electrophysiological experiments reinforced the role of DAGL in short-term plasticity of synaptic activity in the hippocampus.

6. DAGL-dependent eCB signalling regulates synaptic function.

The above genetic and pharmacological studies have established that DAGLα plays a central role in regulating 2-AG, AA and prostaglandin synthesis in the brain and other tissues. However, they do not directly address the role of DAGLα or DAGLβ in eCB signalling pathways. 2-AG has a short half-life (Jarai *et al.*, 2000) and a relatively low affinity for the CB_1/CB_2 receptors (Sugiura and Waku, 2000),

thus it is reasonable to assume that “on-demand” physiological eCB signalling via the DAGLs will require their close proximity to the cannabinoid receptors.

It has long been recognised that AEA and/or 2-AG serve as retrograde synaptic messengers throughout the nervous system (Alger, 2012). DAGLα is well placed to be the key enzyme in this pathway as its expression in neurons is restricted to dendrites in the adult brain (Bisogno *et al.*, 2003) with high resolution imaging studies showing enrichment in all postsynaptic sites that juxtapose CB_1-positive synaptic terminals (Katona *et al.*, 2006; Uchigashima *et al.*, 2007; Yoshida *et al.*, 2006; Yoshida *et al.*, 2011). Live cell imaging has recently shown DAGLα to be localised to an endosomal compartment that can shuttle the enzyme to and from the surface of the dendritic spine in dynamic manner (Zhou *et al.*, 2016). It follows that 2-AG might be made within endosomes possibly providing a regulated vesicular release mechanism to be considered alongside the current model that posits passive diffusion from the cell membrane. 2-AG mobilisation at synapses is established to be triggered by excitatory neurotransmitters (glutamate, acetylcholine, dopamine) acting on their respective postsynaptic receptors and involves a G-protein and/or calcium stimulated synthesis of substrate by a PLCβ, however there also appears to be an additional calcium-dependent step operating independently of substrate synthesis (Ohno-Shosaku and Kano, 2014). One possibility is that calcium (and other signals) might stimulate phosphorylation and opening of a regulatory lid on DAGLα to facilitate substrate access to the catalytic site (Reisenberg *et al.*, 2012).

Depolarisation induced suppression of inhibition or excitation (DSI/DSE) is perhaps the best characterised eCB function in the adult brain (Ohno-Shosaku and Kano, 2014). Numerous studies on DAGLα knockout mice have identified it as the pivotal enzyme controlling this phasic form of synaptic plasticity throughout the nervous system (Gao *et al.*, 2010; Tanimura *et al.*, 2010; Yoshino *et al.*, 2011). In contrast there is little if any effect of knocking out DAGLβ on this almost ubiquitous form of synaptic plasticity (Gao *et al.*, 2010; Tanimura *et al.*, 2010). It is now clear that DAGLα generates the signalling pool of 2-AG that acts on presynaptic CB_1 receptors to dampen the release

of excitatory and inhibitory neurotransmitters to regulate phasic and tonic forms of synaptic plasticity (Lee *et al.*, 2015; Ramikie *et al.*, 2014; Younts and Castillo, 2014). This in turn impacts on a myriad of fundamental behaviours including appetite, pain and cognition (Alger and Kim, 2011). Indeed, it is noteworthy that DAGLα and CB_1 receptor KO mice share the same disrupted phenotypes (Powell *et al.*, 2015) implicating 2-AG as the "workhorse" eCB in the nervous system. The adverse psychiatric consequences of inhibiting CB_1 function in humans revealed in clinical trials for obesity with a CB_1 antagonist (Moreira and Crippa, 2009) are also likely to be consequential to the disruption of 2-AG signalling at synapses as DAGLα KO mice have been used to demonstrate a causative link between synaptic eCB signalling and anxiety and depressive behaviours (Jenniches *et al.*, 2016; Shonesy *et al.*, 2014). In addition, dysfunction of tonic 2-AG signalling appears to play a key and possible reversible role in autism (Anderson *et al.*, 2015; Busquets-Garcia *et al.*, 2013; Foldy *et al.*, 2013; Jung *et al.*, 2012; Kerr *et al.*, 2013). Importantly, pharmacological modulation of 2-AG signalling is being investigated as a treatment for major disorders including depression, pain, obesity and neurodegeneration (Kohnz and Nomura, 2014).

7. Release and reuptake of endocannabinoids

The mechanism(s) by which endocannabinoids are released from cells and subsequently cleared from the extracellular space have been a matter of controversy, the key question being whether or not there exists an as yet unidentified plasma membrane transporter for these lipids (for reviews of the data from proponents and opponents, respectively, of the concept of a plasma endocannabinoid membrane transporter, see (Fowler, 2013; Nicolussi and Gertsch, 2015). It is possible that certain cell types may utilise proteins like pannexin-1 for the release of endocannabinoids (Hill *et al.*, 2015), but more data are needed. What is clear is that endocannabinoids (and NAEs) can be cleared from the extracellular space, and that there are intracellular proteins with other primary functions that can shuttle these lipids, once they have crossed the plasma membrane, to their intracellular

locations, be it the TRPV1 receptor (for AEA), PPARα (for palmitoylethanolamide, PEA), or their catabolic enzymes (Kaczocha *et al.*, 2009; Oddi *et al.*, 2009).

The best characterised of these shuttling proteins with respect to endocannabinoids are FAPB 5 and 7. Crystallographic studies have indicated that AEA binds, albeit with a lower affinity than arachidonic acid, to FABP5 with its lipophilic chain forming a loop in the substrate-binding pocket of this protein and with hydrogen bonding between the hydroxyl group of the ethanolamine to a Tyr^{131} residue. The interaction of FABP5 with 2-AG is largely similar, albeit with looser hydrophobic contacts and more hydrogen bonding (Sanson *et al.*, 2014). Pharmacological inhibition of FABP5/7 reduces uptake of AEA into wild-type HeLa cells and by other FABP5-expressing cell types, but does not inhibit AEA uptake by FABP5 shRNA-expressing HeLa cells (Berger *et al.*, 2012; Bjorklund *et al.*, 2014). Mice lacking FABP5 and 7 show increased brain levels of AEA, PEA and oleoylethanolamide (OEA), but not 2-AG (Kaczocha *et al.*, 2015). A fatty acid amide hydrolase-like transporter protein has also been proposed as an intracellular carrier protein, but its existence has been disputed (Fu *et al.*, 2012; Leung *et al.*, 2013).

8. Enzymatic hydrolysis of AEA and related NAEs

The enzymes responsible for the hydrolysis of AEA and related NAEs to their respective long-chain fatty acids are fatty acid amide hydrolase (FAAH) and *N*-acylethanolamine-hydrolysing acid amidase (NAAA). FAAH is a membrane-bound homodimer belonging to the serine hydrolase family of enzymes and with a wide substrate-selectivity including *N*-acylethanolamines, *N*-acylamides and *N*-acyltaurines (Bachur and Udenfriend, 1966; Boger *et al.*, 2000; Deutsch and Chin, 1993; McKinney and Cravatt, 2006; Schmid *et al.*, 1995). Molecular dynamics and crystallographic studies have indicated that the substrate enters the active site via a membrane access channel, where a triad of residues (Ser^{241}, Ser^{217} and Lys^{142}) are necessary for hydrolysis to occur, and where additional residues (Phe^{432} and Trp^{531}) may act as a "dynamic paddle" to ensure correct substrate orientation and localisation (Bracey *et al.*, 2002; Mileni *et al.*, 2008; Palermo *et al.*, 2015). In the hippocampus,

FAAH has a post-synaptic location, consistent with an orthograde release of AEA synthesized presynaptically by NAPE-PLD and in contrast to the retrograde release of 2-AG (Gulyas *et al.*, 2004; Nyilas *et al.*, 2008).

In mammals, but not rodents, a second FAAH (FAAH-2) has been found, with a preferential localisation within lipid rafts rather than the endoplasmic reticulum (the location of FAAH) (Kaczocha *et al.*, 2010; Wei *et al.*, 2006). The two FAAH species have different relative hydrolysis rates for substrates. Thus at pH 9 (in the region of the pH optimum for both FAAH enzymes), the rate of hydrolysis of 100 μM AEA, OEA, PEA, and *N*-oleoyl taurine, respectively, relative to that of oleamide was 1.75, 0.58, 0.22 and 0.77 for human FAAH transfected into COS-7 cells, and 0.055, 0.23, 0.024 and not detected for *N*-terminal FLAG-tagged FAAH-2 transfected into these cells (Wei *et al.*, 2006). The sensitivity of the two enzymes to inhibition is also different, with FAAH-2 being more sensitive than FAAH(-1) to inhibition by the carbamate inhibitor URB597 and the α-ketoheterocycle OL-135, whilst the reverse is true for the piperazinyl phenyl urea compound JNJ-1661010 and the isoflavone biochanin A (Karbarz *et al.*, 2009; Thors *et al.*, 2010b; Wei *et al.*, 2006) (Table 1).

In addition to FAAH, NAEs are hydrolysed by NAAA, an enzyme with a pH optimum around pH 5, and with a preference for the unsaturated substrates over the saturated substrates (PEA > myristoylethanolamide > stearoylethanolamide (SEA) ≃ OEA > linoleoylethanolamide > AEA (Ueda *et al.*, 2001). The enzyme has its highest distribution in the lung where it is localised to the lysosomes of macrophages (Tsuboi *et al.*, 2007; Ueda *et al.*, 2001). NAAA is a glycoprotein with sequence homology to acid ceramidase rather than FAAH (Tsuboi *et al.*, 2005).

9. Enzymatic hydrolysis of 2-AG and related monoacylglycerols

Multiple enzyme activities have been identified to be capable of hydrolysing 2-AG in vitro, including monoacylglycerol lipase (MAGL) (Dinh *et al.*, 2002a), ABHD2 (Miller *et al.*, 2016), ABHD6 (Blankman *et al.*, 2007; Navia-Paldanius *et al.*, 2012), ABHD12 (Navia-Paldanius *et al.*, 2012), neuropathy target

esterase (van Tienhoven *et al.*, 2002) and carboxylesterase 1 (Xie *et al.*, 2010). However, it's clear that MAGL performs the bulk of 2-AG hydrolysis in most tissues given the impact of gene disruption on 2-AG levels (Pan *et al.*, 2011; Zhong *et al.*, 2011). In addition, a functional, activity-based protein profiling approach using mouse brain membranes indicated that MAGL accounted for the majority of 2AG hydrolysis (~85 %), while ABHD6 and ABHD12 accounted for the remainder (Blankman *et al.*, 2007). Despite this predominance of MAGL, it's quite likely that some of these enzymes have influential roles on 2-AG turnover, albeit in niche locations, both at tissue and cellular levels.

Aside from a common substrate, many of these enzymes have a common structure in that they are alpha/beta hydrolases. These are a large family of enzymes characterised by a primary sequence motif known as an α/β hydrolase fold domain – an extended organisation of α-helices and β-sheets – where the most recognisable family member is acetylcholinesterase (Thomas *et al.*, 2014). This family also includes the diacylglycerol lipases (DAGL-α and β), epoxide hydrolases, and lipoprotein and endothelial lipases.

MAGL: MAGL is a 303-aa protein (~33 kDa) serine hydrolase, with a characteristic triad of a charge relay of Asp^{239}-His^{269} around the catalytic nucleophile Ser^{122}. There are no identifiable transmembrane (TM) domains, but the enzyme is found associated with both cytosolic and particulate compartments (Ghafouri *et al.*, 2004). In adipose tissue, MAGL performs the final step in lipolysis, hydrolysing monoacylglycerols to produce free fatty acid and glycerol; this role was identified over 30 years before a role in endocannabinoid turnover was suggested (Vaughan *et al.*, 1964). In some tissues, such as the brain, it appears that MAGL-evoked arachidonic acid is the predominant route for prostaglandin production (Nomura *et al.*, 2011) and above). MAGL shows hydrolytic preference for 2-AG, but not AEA (Dinh *et al.*, 2002a; Dinh *et al.*, 2002b), but with little specificity between acylglycerols (Ghafouri *et al.*, 2004; Vandevoorde *et al.*, 2005).

ABHD2: The *ABHD2* gene encodes a 425-aa protein (~48 kDa) serine hydrolase (Ser^{207}-Asp^{345}-His^{376}). A single TM is predicted at 10-30, with an extracellular catalytic domain. Glycosylation is predicted at

Asn^{136} and Asn^{410}, while a single nucleotide polymorphism has been described (Arg^{253}Gln). ABHD2 was initially cloned as one of three α/β-hydrolases from mouse lung (Edgar and Polak, 2002). The mRNA was expressed in mouse brain, heart, kidney, liver, lungs, skeletal muscle and spleen, but was highest in testes (Edgar and Polak, 2002). Gene trap analysis in mice suggested an expression in smooth muscle (vascular and non-vascular), but not skeletal muscle (Miyata *et al.*, 2005). The mice lacking *abhd2* appeared to have normal vasculature, but explants were more proliferative and cuff placement resulted in greater intimal hyperplasia. A variety of links with potential therapeutic indications have been reported. For example, in unstable angina (Miyata *et al.*, 2008), hepatitis B virus propagation (Ding *et al.*, 2008), age-related pulmonary emphysema (Jin *et al.*, 2009), ovarian (Yamanoi *et al.*, 2016) and prostate cancer (Obinata *et al.*, 2016). ABHD2 is reported to have progesterone-dependent monoacylglycerol lipase activity and to be inhibited by MAFP (Miller *et al.*, 2016). In addition, ABHD2 appears to be able to hydrolyse triacylglycerols (Naresh Kumar *et al.*, 2016).

ABHD6: ABHD6 is a 337-aa protein (~38 kDa) serine hydrolase (Ser^{148}-Asp^{278}-His^{306}). A single TM is predicted at 9-29, with an intracellular catalytic domain. ABHD6 is a monoacylglycerol hydrolase (Navia-Paldanius *et al.*, 2012), with little selectivity for 1- compared to 2-acylglycerols (Thomas *et al.*, 2013), and possibly bis(monoacylglycerol)phosphate hydrolase (Pribasnig *et al.*, 2015). Antisense knockdown of ABHD6 led to accumulation of numerous phospholipids and lysophospholipids in mouse liver implying a role in glycerophospholipid turnover in the liver (Thomas *et al.*, 2013). ABHD6 has been implicated in diabetes/metabolic disorder (Zhao *et al.*, 2016; Zhao *et al.*, 2015), inflammation (Alhouayek *et al.*, 2013) and epilepsy (Naydenov *et al.*, 2014).

ABHD12: ABHD12 is a 398-aa protein (~45 kDa) serine hydrolase (Ser^{246}-Asp^{333}-His^{372}). A single TM is predicted at 75-95, with an extracellular catalytic domain. ABHD12 is a monoacylglycerol hydrolase (Navia-Paldanius *et al.*, 2012), but may also regulate lysophosphatidylserine levels (Kamat *et al.*,

2015). Loss-of-function mutations in ABHD12 are associated with a disorder known as PHARC (polyneuropathy, hearing loss, ataxia, retinitis pigmentosa, and cataracts) (Fiskerstrand *et al.*, 2010).

10 Pharmacological inhibitors of monoacylglycerol hydrolase activities

There is a tantalising indication for potential therapeutic exploitation of these enzymes, but the realisation of this potential is absolutely dependent on the provision of new information on the distribution of these enzymes and the identification of their 'natural' substrates. In addition, generation of cheap, reproducible high-throughput screening assays will facilitate novel drug discovery to allow future pharmacological investigation, thereby enhancing an understanding of the role/s of these enzymes in pathological circumstances, leading ultimately to their therapeutic exploitation.

MAGL inhibitors: For MAGL activity, a number of useful tools are commercially available. JZL184 is a relatively selective (certainly compared to FAAH) irreversible MAGL inhibitor with a pIC_{50} value of 8.1-8.4 (Chang *et al.*, 2012; Long *et al.*, 2009) in human and mouse. JZL184 is slightly less potent against rat MAGL, with a pIC_{50} value of 6.5-7.1 (Woodhams *et al.*, 2012). In a separate study, a similar discrepancy between mouse and rat potency of JZL184 was also noted, although with slightly lower potencies (Aaltonen *et al.*, 2013). It's likely that these lab-to-lab differences reflect the irreversible nature of the inhibitor, where conditions such as substrate concentration, pH, protein content and (probably most influential) pre-incubation period are major sources of variation.

JJKK048 is even more potent and selective as a MAGL inhibitor, with a sub-nanomolar potency (pIC_{50} value of 9.4-9.7), with essentially equivalent potency at mouse, rat or human enzymes (Aaltonen *et al.*, 2013). Although substantially less potent (pIC_{50} 6.6), Compound 21 (Hernandez-Torres *et al.*, 2014) represents a reversible MAGL inhibitor, which is active *in vivo*.

The use of JZL184 in vivo produced effects similar to those obtained using THC or synthetic CB_1 cannabinoid agonists (Long *et al.*, 2009), indicating that there may be psychoactive limiting factors

for therapeutic application of MAGL inhibitors. Despite this, there are reports of anxiolysis (Sciolino *et al.*, 2011), reduction in pain behaviours (Aaltonen *et al.*, 2016; Woodhams *et al.*, 2012) and nausea-like behaviours (Sticht *et al.*, 2012), as well as neuroprotection in a model of Parkinson's disease (Fernandez-Suarez *et al.*, 2014).

ABHD6 inhibitors: WWL70 was the first described selective ABHD6 inhibitor; screening mouse brain membranes using the activity-based protein profiling system generated a pIC_{50} value of ~7.2 (Li *et al.*, 2007). From the same group, WWL123 is a similarly structured inhibitor with a slightly less potent profile (Bachovchin *et al.*, 2010). In addition, KT185 has been described as an orally-active ABHD6-selective inhibitor (Hsu *et al.*, 2013).

In vivo administration of WWL70 was neuroprotective in a mouse model of traumatic brain injury (Tchantchou and Zhang, 2013), and also alleviated symptoms in a model of multiple sclerosis in a manner dependent on CB_2 cannabinoid receptors (Wen *et al.*, 2015). WWL70 also protected mice from the weight gain associated with a high fat diet (Thomas *et al.*, 2013), in a manner consistent with the involvement of the nuclear hormone receptors PPARs (Zhao *et al.*, 2016) (see O'Sullivan and Pistis, this volume). WWL123 has also been shown to be effective in vivo, by reducing seizures in an epilepsy model (Naydenov *et al.*, 2014).

ABHD12 inhibitors: Currently, there is no selective inhibitor that targets ABHD12. However, it is possible to use a process of elimination to identify involvement of ABHD12 in monoacylglycerol hydrolase activity. Thus, ABHD12 is relatively insensitive to the MAGL inhibitors JZL184 or JJKK-048, the ABHD6 inhibitor WWL70 or the DAGL inhibitor RHC80267, but may be inhibited by the non-selective serine hydrolase inhibitor MAFP and THL (more widely known as an inhibitor of DAGL, see above, and pancreatic lipase) (Blankman *et al.*, 2007).

11 Oxidative metabolism of endocannabinoids

Given that AEA and 2-AG have an arachidonoyl side-chain, it is not surprising that they share some of the metabolic pathways of arachidonic acid, or that many of the products of these pathways have been shown to have biological actions of their own. The main enzymes involved are summarised below. Aficionados of chemical structures are referred to Urquhart et al. (2015), where these catabolic pathways are well illustrated.

Cyclooxygenase (COX) -2 pathway. Both AEA and 2-AG are metabolised by COX-2, but not by COX-1 (due to a narrower active site), to yield prostaglandin ethanolamindes (PG-EAs) and prostaglandin glyceryl esters (PG-GEs), respectively (Kozak *et al.*, 2000; Yu *et al.*, 1997). COX-2 acts as a functional heterodimer, whereby allosteric binding to one site affects the catalytic activity at the other, and crystallographic studies of COX-2 have indicated that 1-AG binds slightly differently to the two sites (Mitchener *et al.*, 2015; Vecchio and Malkowski, 2011). $PGF_{2\alpha}$-EA formation from AEA proceeds via the PGF-EA synthase catalysed reduction of PGH_2-EA (Moriuchi *et al.*, 2008; Yang *et al.*, 2005), and produces its biological effects, including an increased firing of spinal cord nociceptive neurons, as a result of an interaction with a heterodimeric receptor that is not responsive to $PGF_{2\alpha}$ (Gatta *et al.*, 2012; Woodward *et al.*, 2013). Most of the work on the properties of PG-GEs have been undertaken using the more stable 1,3-regioisomers, but a picture is emerging whereby both PG-EA and PG-GE compounds can affect inflammation, with both pro- and anti-inflammatory effects being reported (review, see (Alhouayek and Muccioli, 2014)). The stable PG-EA analogue bimatoprost is clinically used for the treatment of glaucoma (see (Woodward *et al.*, 2013)). Relatively little is known about the metabolism of PG-EAs, but a major metabolic pathway for PG-GEs is hydrolysis to PG catalysed by several enzymes, lysophospholipase A_2 playing a major role in this respect (Manna *et al.*, 2014).

Lipoxygenase (LOX) pathways. Less work has been undertaken on LOX-catalysed hydroperoxidation of the endocannabinoids, but both AEA and 2-AG are substrates of 12- and 15-LOX to produce products that are biologically active either at CB receptors or at peroxisome proliferator-active

receptor α (Edgemond *et al.*, 1998; Hampson *et al.*, 1995; Kozak *et al.*, 2002; Moody *et al.*, 2001; Ueda *et al.*, 1995; van der Stelt *et al.*, 2002). In addition, the 15-LOX product of AEA can be metabolised by glutathione transferases to form cysteinyl-containing metabolites ("eoxamides", in analogy with the eoxins produced from arachidonic acid) (Forsell *et al.*, 2012). The biological properties of the eoxamides is not known.

CYP450 pathways. In 1995, Bornheim and colleagues reported that the incubation of AEA with liver microsomes in the presence of NADPH resulted in the production of several compounds, the levels of which could be induced following pretreatment of the mice with CYP450 enzyme inducers (Bornheim *et al.*, 1995). Subsequent work mainly by Hollenberg and colleagues have identified CYP3A4, CYP2D6, CYP4F2 and CYP2J2-derived AEA metabolites, and shown that the 5,6-epoxide metabolite of AEA is a reasonably potent and selective CB_2 receptor agonist (McDougle *et al.*, 2014; Snider *et al.*, 2007; Snider *et al.*, 2009; Snider *et al.*, 2008; Walker *et al.*, 2016). CYP-derived metabolites of 2-AG with activity towards CB receptors have also been described (Chen *et al.*, 2008; McDougle *et al.*, 2014).

12 Integrating the catabolism of the endocannabinoids and related NAEs in health and disease

From the above, it is clear that there are a multiplicity of synthetic and degradative pathways for the endocannabinoids. At first sight, this would suggest that a "point block" (i.e. a selective inhibition or genetic deletion of one of the metabolic enzymes) would be expected to have a limited effect (since the endocannabinoid would merely find another metabolic route), unless the enzyme in question was predominant. Indeed, under extreme conditions (such as genetic deletion), additional metabolic pathways in addition to those described above may come into play (Mulder and Cravatt, 2006). As pointed out previously, deletion of NAPE-PLD reduces, but does not completely block, AEA production in the brain (Leishman *et al.*, 2016; Leung *et al.*, 2006; Tsuboi *et al.*, 2011). In this section, the effects of point blocks of the catabolic enzyme FAAH is considered.

Early studies using FAAH inhibitors and $FAAH^{-/-}$ mice demonstrated that, providing enzyme activity was sufficiently inhibited, brain levels of AEA were dramatically increased (Cravatt *et al.*, 2001; Kathuria *et al.*, 2003). This is not a~~n~~ universal finding: in lipopolysaccharide + interferon γ- activated macrophages, for example, hydrolysis of exogenous AEA is totally blocked by the FAAH inhibitor URB597, but the endogenous levels of AEA are, at best, only modestly affected (Gouveia-Figueira *et al.*, 2015a). Studies investigating the effects of FAAH inhibition and/or genetic deletion on AEA and related NAE levels either in different brain regions or in different parts of the body have been undertaken, but these tend to suffer from statistical issues such as small sample sizes, lack of consideration of unequal variances between groups and, not least, lack of compensation for multiple significance testing. This does not impact upon the large-scale changes reported, but more modest changes may be either over-interpreted or missed completely.

An alternative approach is to look at the organism as a whole. In a study from 2014, a three compartment model (brain, plasma and rest of body) was used to simulate the changes over time in plasma AEA, PEA, OEA and linoleoyl ethanolamide levels in humans treated with the FAAH inhibitor PF-04457845. They could simulate the observed data well, but only if the model included an FAAH-independent clearance (Benson *et al.*, 2014). The authors suggested that NAAA may be a suitable candidate for this additional clearance, on the basis that PEA was also encompassed by this finding. Certainly, inhibition of NAAA increases PEA and OEA levels in mouse lungs (Ribeiro *et al.*, 2015), but it is unclear as to whether the more limited organ distribution of this enzyme is sufficient to explain the data.

This somewhat complex system becomes even more convoluted when patients with diseases potentially tractable to treatment with inhibitors of AEA / NAE hydrolysis are considered, such as patients with pain and inflammation (for review for FAAH and pain, see (Fowler, 2015); for inflammation and NAAA, see (Alhouayek *et al.*, 2015; Solorzano *et al.*, 2009) for examples in experimental animals). In this case, the situation is complicated by the presence and influence of

cytokines and other factors that can dramatically affect the relative expression of the catabolic enzymes. Thus, in addition to a large induction in COX-2 activity, cytokines like the interleukins IL-4 and IL-10 increase FAAH activity, whilst IL-12 and interferon-γ reduce it (Maccarrone *et al.*, 2001b; Thors *et al.*, 2010a). In unstimulated RAW264.7 macrophages, the mRNA expression of NAAA is about 160-fold higher than that of FAAH. However, upon activation with 24 h of treatment with lipopolysaccharide + interferon-γ, NAAA mRNA is reduced five-fold, whilst FAAH mRNA is increased fourfold (Gabrielsson et al., submitted; see Figure 3). Whilst these changes are less than the 1400-fold induction of COX-2 in the cells, the treatment clearly changes the balance of AEA catabolic enzymes in the cells. Further, changes are likely to be cell-specific: in lymphocytes, for example, lipopolysaccharide reduces rather than increases FAAH expression (Maccarrone *et al.*, 2001a). Such changes in the relative expression of the catabolic enzymes may provide an explanation for the loss of effect of the FAAH inhibitor URB597 towards increasing hindpaw AEA, OEA and 2-AG levels in rats with neuropathic pain following spinal nerve ligation (Jhaveri *et al.*, 2006), and even could be a contributing factor to the failure of the FAAH inhibitor PF-04457845 in osteoarthritis (Huggins *et al.*, 2012). Modelling such changes is by no means easy, but a simple model can be constructed from the known K_m and k_{cat} values for FAAH and COX-2 for given AEA concentrations in the vicinity of the enzymes (Figure 3). Add to this a) the ability of endogenous lipids such as cholesterol, phosphatidylcholine, dihydrolipoic acid and AEA-derived LOX metabolites to influence the activity of FAAH and/or NAAA (Dainese *et al.*, 2014; Tai *et al.*, 2012; van der Stelt *et al.*, 2002) and b) differences in CYP3A4, 2BC and 2D6 polymorphisms with respect to their ability to metabolise AEA (Pratt-Hyatt *et al.*, 2010; Sridar *et al.*, 2011) and it becomes clear that the potential for differences in catabolism of endocannabinoids between cells, organs, individuals and between healthy volunteers and patients, becomes considerable.

The aim of this review was to discuss the turnover of endocannabinoids rather than the pharmacology of the system, but it is clear from the above discussion that “point block” may not be optimal in cases where alternative catabolic pathways are present. An interesting alternative is the

design of compounds attacking more than one catabolic pathway. One possibility is to target both COX-2 and FAAH. Two approaches have been taken: one is to start from the modest FAAH inhibitory potency of COX inhibitors such as ibuprofen and flurbiprofen, and to optimise the former without compromising the latter. This has led to the identification of (*R*)-2-(2-fluorobiphenyl-4-yl)-*N*-(3-methylpyridin-2-yl)propanamide, a mixed-type reversible inhibitor of FAAH and substrate-selective inhibitor of COX-2 (Gouveia-Figueira *et al.*, 2015b; Karlsson *et al.*, 2015). An alternative approach is to link a COX inhibitor (flurbiprofen) to a carbamate FAAH inhibitor with a chemical bridge (Migliore *et al.*, 2016; Sasso *et al.*, 2015). It will be interesting to see whether such compounds prove to be useful in conditions where both COX-2 and FAAH play central roles in AEA catabolism.

13 Conclusion

There is an attractive complexity associated with the network of enzymes involved in the synthesis, hydrolysis and transformation of endocannabinoids. The various genetic models and pharmacological tools are beginning to allow dissection of the patho/physiological roles of these enzymes, which paves the way for future exploitation in a clinical environment.

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Conflict of interest

The authors have no conflict of interest to relate.

References

Aaltonen N, Kedzierska E, Orzelska-Gorka J, Lehtonen M, Navia-Paldanius D, Jakupovic H, Savinainen J, Nevalainen T, Laitinen JT, Parkkari T, Gynther M (2016). In vivo characterization of the ultrapotent monoacylglycerol lipase inhibitor {4-[bis-(benzo[d][1,3]dioxol-5-yl)methyl]-piperidin-1-yl}(1H-1,2,4-triazol-1-yl)m ethanone (JJKK-048). *J Pharmacol Exp Ther*, **359**: 62 - 72.

Aaltonen N, Savinainen JR, Ribas CR, Ronkko J, Kuusisto A, Korhonen J, Navia-Paldanius D, Hayrinen J, Takabe P, Kasnanen H, Pantsar T, Laitinen T, Lehtonen M, Pasonen-Seppanen S, Poso A, Nevalainen T, Laitinen JT (2013). Piperazine and piperidine triazole ureas as ultrapotent and highly selective inhibitors of monoacylglycerol lipase. *Chem Biol*, **20**: 379 - 390.

Alger BE (2012). Endocannabinoids at the synapse a decade after the Dies Mirabilis (29 March 2001): what we still do not know. *J Physiol*, **590**: 2203 - 2212.

Alger BE, Kim J (2011). Supply and demand for endocannabinoids. *Trends Neurosci*, **34**: 304 - 315.

Alhouayek M, Bottemanne P, Subramanian KV, Lambert DM, Makriyannis A, Cani PD, Muccioli GG (2015). N-Acylethanolamine-hydrolyzing acid amidase inhibition increases colon N-palmitoylethanolamine levels and counteracts murine colitis. *FASEB J*, **29**: 650 - 661.

Alhouayek M, Masquelier J, Cani PD, Lambert DM, Muccioli GG (2013). Implication of the anti-inflammatory bioactive lipid prostaglandin D2-glycerol ester in the control of macrophage activation and inflammation by ABHD6. *Proc Natl Acad Sci U S A*, **110**: 17558 - 17563.

Alhouayek M, Muccioli GG (2014). COX-2-derived endocannabinoid metabolites as novel inflammatory mediators. *Trends Pharmacol Sci*, **35**: 284 - 292.

Allen AC, Gammon CM, Ousley AH, McCarthy KD, Morell P (1992). Bradykinin stimulates arachidonic acid release through the sequential actions of an sn-1 diacylglycerol lipase and a monoacylglycerol lipase. *J Neurochem*, **58**: 1130 - 1139.

Amantea D, Spagnuolo P, Bari M, Fezza F, Mazzei C, Tassorelli C, Morrone LA, Corasaniti MT, Maccarrone M, Bagetta G (2007). Modulation of the endocannabinoid system by focal brain ischemia in the rat is involved in neuroprotection afforded by 17 β-estradiol. *FEBS J*, **274**: 4464 - 4475.

Anderson GR, Aoto J, Tabuchi K, Foldy C, Covy J, Yee AX, Wu D, Lee SJ, Chen L, Malenka RC, Sudhof TC (2015). β-Neurexins Control Neural Circuits by Regulating Synaptic Endocannabinoid Signaling. *Cell*, **162**: 593 - 606.

Araki M, Ohshima N, Aso C, Konishi A, Obinata H, Tatei K, Izumi T (2016). Enzymatic characterization of recombinant rat DDHD2: a soluble diacylglycerol lipase. *J Biochem*, **160**: 269 - 279.

Bachovchin DA, Ji T, Li W, Simon GM, Blankman JL, Adibekian A, Hoover H, Niessen S, Cravatt BF (2010). Superfamily-wide portrait of serine hydrolase inhibition achieved by library-versus-library screening. *Proc Natl Acad Sci U S A*, **107**: 20941 - 20946.

Bachur NR, Udenfriend S (1966). Microsomal synthesis of fatty acid amides. *J Biol Chem*, **241**: 1308 - 1313.

Baggelaar MP, Chameau PJ, Kantae V, Hummel J, Hsu KL, Janssen F, van der Wel T, Soethoudt M, Deng H, den Dulk H, Allara M, Florea BI, Di Marzo V, Wadman WJ, Kruse CG, Overkleeft HS, Hankemeier T, Werkman TR, Cravatt BF, van der Stelt M (2015). Highly Selective, Reversible Inhibitor Identified by Comparative Chemoproteomics Modulates Diacylglycerol Lipase Activity in Neurons. *J Am Chem Soc*, **137**: 8851 - 8857.

Bazinet RP, Laye S (2014). Polyunsaturated fatty acids and their metabolites in brain function and disease. *Nat Rev Neurosci*, **15**: 771 - 785.

Bell RL, Kennerly DA, Stanford N, Majerus PW (1979). Diglyceride lipase: a pathway for arachidonate release from human platelets. *Proc Natl Acad Sci U S A*, **76**: 3238 - 3241.

Benson N, Metelkin E, Demin O, Li GL, Nichols D, van der Graaf PH (2014). A systems pharmacology perspective on the clinical development of fatty acid amide hydrolase inhibitors for pain. *CPT Pharmacometrics Syst Pharmacol*, **3**: e91 -

Berdyshev EV, Schmid PC, Krebsbach RJ, Schmid HH (2001). Activation of PAF receptors results in enhanced synthesis of 2-arachidonoylglycerol (2-AG) in immune cells. *FASEB J*, **15**: 2171 - 2178.
Berger WT, Ralph BP, Kaczocha M, Sun J, Balius TE, Rizzo RC, Haj-Dahmane S, Ojima I, Deutsch DG (2012). Targeting fatty acid binding protein (FABP) anandamide transporters - a novel strategy for development of anti-inflammatory and anti-nociceptive drugs. *PLoS One*, **7**: e50968 -
Bisogno T, Howell F, Williams G, Minassi A, Cascio MG, Ligresti A, Matias I, Schiano-Moriello A, Paul P, Williams EJ, Gangadharan U, Hobbs C, Di Marzo V, Doherty P (2003). Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. *J Cell Biol*, **163**: 463 - 468.
Bjorklund E, Blomqvist A, Hedlin J, Persson E, Fowler CJ (2014). Involvement of fatty acid amide hydrolase and fatty acid binding protein 5 in the uptake of anandamide by cell lines with different levels of fatty acid amide hydrolase expression: a pharmacological study. *PLoS One*, **9**: e103479 -
Blankman JL, Simon GM, Cravatt BF (2007). A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol. *Chem Biol*, **14**: 1347 - 1356.
Boger DL, Fecik RA, Patterson JE, Miyauchi H, Patricelli MP, Cravatt BF (2000). Fatty acid amide hydrolase substrate specificity. *Bioorg Med Chem Lett*, **10**: 2613 - 2616.
Bornheim LM, Kim KY, Chen BL, Correia MA (1995). Microsomal cytochrome P450-mediated liver and brain anandamide metabolism. *Biochem Pharmacol*, **50**: 677 - 686.
Bracey MH, Hanson MA, Masuda KR, Stevens RC, Cravatt BF (2002). Structural adaptations in a membrane enzyme that terminates endocannabinoid signaling. *Science*, **298**: 1793 - 1796.
Busquets-Garcia A, Gomis-Gonzalez M, Guegan T, Agustin-Pavon C, Pastor A, Mato S, Perez-Samartin A, Matute C, de la Torre R, Dierssen M, Maldonado R, Ozaita A (2013). Targeting the endocannabinoid system in the treatment of fragile X syndrome. *Nat Med*, **19**: 603 - 607.
Cadas H, Gaillet S, Beltramo M, Venance L, Piomelli D (1996). Biosynthesis of an endogenous cannabinoid precursor in neurons and its control by calcium and cAMP. *J Neurosci*, **16**: 3934 - 3942.
Chang JW, Niphakis MJ, Lum KM, Cognetta AB, III, Wang C, Matthews ML, Niessen S, Buczynski MW, Parsons LH, Cravatt BF (2012). Highly Selective Inhibitors of Monoacylglycerol Lipase Bearing a Reactive Group that Is Bioisosteric with Endocannabinoid Substrates. *Chem Biol*, **19**: 579 - 588.
Chen JK, Chen J, Imig JD, Wei S, Hachey DL, Guthi JS, Falck JR, Capdevila JH, Harris RC (2008). Identification of novel endogenous cytochrome P450 arachidonate metabolites with high affinity for cannabinoid receptors. *J Biol Chem*, **283**: 24514 - 24524.
Cravatt BF, Demarest K, Patricelli MP, Bracey MH, Giang DK, Martin BR, Lichtman AH (2001). Supersensitivity to anandamide and enhanced endogenous cannabinoid signaling in mice lacking fatty acid amide hydrolase. *Proc Natl Acad Sci U S A*, **98**: 9371 - 8971.
Dainese E, De Fabritiis G, Sabatucci A, Oddi S, Angelucci CB, Di Pancrazio C, Giorgino T, Stanley N, Del Carlo M, Cravatt BF, Maccarrone M (2014). Membrane lipids are key modulators of the endocannabinoid-hydrolase FAAH. *Biochem J*, **457**: 463 - 472.
Deutsch DG, Chin SA (1993). Enzymatic synthesis and degradation of anandamide, a cannabinoid receptor agonist. *Biochem Pharmacol*, **46**: 791 - 796.
Di Marzo V (2011). Endocannabinoid signaling in the brain: biosynthetic mechanisms in the limelight. *Nat Neurosci*, **14**: 9 - 15.
Ding XR, Yang J, Sun DC, Lou SK, Wang SQ (2008). Whole genome expression profiling of hepatitis B virus-transfected cell line reveals the potential targets of anti-HBV drugs. *Pharmacogenomics J*, **8**: 61 - 70.
Dinh TP, Carpenter D, Leslie FM, Freund TF, Katona I, Sensi SL, Kathuria S, Piomelli D (2002a). Brain monoglyceride lipase participating in endocannabinoid inactivation. *Proc Natl Acad Sci U S A*, **99**: 10819 - 10824.
Dinh TP, Freund TF, Piomelli D (2002b). A role for monoglyceride lipase in 2-arachidonoylglycerol inactivation. *Chem Phys Lipids*, **121**: 149 - 158.
Edgar AJ, Polak JM (2002). Cloning and tissue distribution of three murine α/β hydrolase fold protein cDNAs. *Biochem Biophys Res Commun*, **292**: 617 - 625.

Edgemond WS, Hillard CJ, Falck JR, Kearn CS, Campbell WB (1998). Human platelets and polymorphonuclear leukocytes synthesize oxygenated derivatives of arachidonylethanolamide (anandamide): their affinities for cannabinoid receptors and pathways of inactivation. *Mol Pharmacol*, **54**: 180 - 188.
Farooqui AA, Taylor WA, Horrocks LA (1984). Separation of bovine brain mono- and diacylglycerol lipases by heparin sepharose affinity chromatography. *Biochem Biophys Res Commun*, **122**: 1241 - 1246.
Fernandez-Suarez D, Celorrio M, Riezu-Boj JI, Ugarte A, Pacheco R, Gonzalez H, Oyarzabal J, Hillard CJ, Franco R, Aymerich MS (2014). The monoacylglycerol lipase inhibitor JZL184 is neuroprotective and alters glial cell phenotype in the chronic MPTP mouse model. *Neurobiol Aging*, **35**: 2603 - 2616.
Fiskerstrand T, H'mida-Ben Brahim D, Johansson S, M'zahem A, Haukanes BI, Drouot N, Zimmermann J, Cole AJ, Vedeler C, Bredrup C, Assoum M, Tazir M, Klockgether T, Hamri A, Steen VM, Boman H, Bindoff LA, Koenig M, Knappskog PM (2010). Mutations in ABHD12 Cause the Neurodegenerative Disease PHARC: An Inborn Error of Endocannabinoid Metabolism. *Am J Hum Genet*, **87**: 410 - 417.
Foldy C, Malenka RC, Sudhof TC (2013). Autism-associated neuroligin-3 mutations commonly disrupt tonic endocannabinoid signaling. *Neuron*, **78**: 498 - 509.
Forsell PK, Brunnstrom A, Johannesson M, Claesson HE (2012). Metabolism of anandamide into eoxamides by 15-lipoxygenase-1 and glutathione transferases. *Lipids*, **47**: 781 - 791.
Fowler CJ (2013). Transport of endocannabinoids across the plasma membrane and within the cell. *FEBS J*, **280**: 1895 - 1904.
Fowler CJ (2015). The Potential of Inhibitors of Endocannabinoid Metabolism for Drug Development: A Critical Review. *Handb Exp Pharmacol*, **231**: 95 - 128.
Franklin A, Parmentier-Batteur S, Walter L, Greenberg DA, Stella N (2003). Palmitoylethanolamide increases after focal cerebral ischemia and potentiates microglial cell motility. *J Neurosci*, **23**: 7767 - 7775.
Fu J, Bottegoni G, Sasso O, Bertorelli R, Rocchia W, Masetti M, Guijarro A, Lodola A, Armirotti A, Garau G, Bandiera T, Reggiani A, Mor M, Cavalli A, Piomelli D (2012). A catalytically silent FAAH-1 variant drives anandamide transport in neurons. *Nat Neurosci*, **15**: 64 - 69.
Gao Y, Vasilyev DV, Goncalves MB, Howell FV, Hobbs C, Reisenberg M, Shen R, Zhang MY, Strassle BW, Lu P, Mark L, Piesla MJ, Deng K, Kouranova EV, Ring RH, Whiteside GT, Bates B, Walsh FS, Williams G, Pangalos MN, Samad TA, Doherty P (2010). Loss of retrograde endocannabinoid signaling and reduced adult neurogenesis in diacylglycerol lipase knock-out mice. *J Neurosci*, **30**: 2017 - 2024.
Garcia-Ovejero D, Arevalo-Martin A, Petrosino S, Docagne F, Hagen C, Bisogno T, Watanabe M, Guaza C, Di Marzo V, Molina-Holgado E (2009). The endocannabinoid system is modulated in response to spinal cord injury in rats. *Neurobiol Dis*, **33**: 57 - 71.
Gatta L, Piscitelli F, Giordano C, Boccella S, Lichtman A, Maione S, Di Marzo V (2012). Discovery of prostamide $F_{2\alpha}$ and its role in inflammatory pain and dorsal horn nociceptive neuron hyperexcitability. *PLoS One*, **7**: e31111 -
Ghafouri N, Tiger G, Razdan RK, Mahadevan A, Pertwee RG, Martin BR, Fowler CJ (2004). Inhibition of monoacylglycerol lipase and fatty acid amide hydrolase by analogues of 2-arachidonoylglycerol. *Br J Pharmacol*, **143**: 774 - 784.
Goncalves MB, Suetterlin P, Yip P, Molina-Holgado F, Walker DJ, Oudin MJ, Zentar MP, Pollard S, Yanez-Munoz RJ, Williams G, Walsh FS, Pangalos MN, Doherty P (2008). A diacylglycerol lipase-CB2 cannabinoid pathway regulates adult subventricular zone neurogenesis in an age-dependent manner. *Mol Cell Neurosci*, **38**: 526 - 536.
Gouveia-Figueira S, Karlsson J, Deplano A, Hashemian S, Svensson M, Fredriksson SM, Congiu C, Onnis V, Fowler CJ (2015a). Characterisation of (R)-2-(2-Fluorobiphenyl-4-yl)-N-(3-Methylpyridin-2-yl)Propanamide as a Dual Fatty Acid Amide Hydrolase: Cyclooxygenase Inhibitor. *PLoS One*, **10**: e0139212 -
Gouveia-Figueira S, Karlsson J, Deplano A, Hashemian S, Svensson M, Fredriksson SM, Congiu C, Onnis V, Fowler CJ (2015b). Characterisation of (R)-2-(2-Fluorobiphenyl-4-yl)-N-(3-Methylpyridin-2-

yl)Propanamide as a Dual Fatty Acid Amide Hydrolase: Cyclooxygenase Inhibitor. *PLoS One*, **10**: e0139212 -
Gulyas AI, Cravatt BF, Bracey MH, Dinh TP, Piomelli D, Boscia F, Freund TF (2004). Segregation of two endocannabinoid-hydrolyzing enzymes into pre- and postsynaptic compartments in the rat hippocampus, cerebellum and amygdala. *Eur J Neurosci*, **20**: 441 - 458.
Hampson AJ, Hill WAG, Zanphillips M, Makriyannis A, Leung E, Eglen RM, Bornheim LM (1995). Anandamide hydroxylation by brain lipoxygenase: metabolite structures and potencies at the cannabinoid receptor. *Biochim Biophys Acta-Lip Lip Met*, **1259**: 173 - 179.
Hansen HH, Ikonomidou C, Bittigau P, Hansen SH, Hansen HS (2001a). Accumulation of the anandamide precursor and other N-acylethanolamine phospholipids in infant rat models of in vivo necrotic and apoptotic neuronal death. *J Neurochem*, **76**: 39 - 46.
Hansen HH, Schmid PC, Bittigau P, Lastres-Becker I, Berrendero F, Manzanares J, Ikonomidou C, Schmid HH, Fernandez-Ruiz JJ, Hansen HS (2001b). Anandamide, but not 2-arachidonoylglycerol, accumulates during in vivo neurodegeneration. *J Neurochem*, **78**: 1415 - 1427.
Hasegawa-Sasaki H (1985). Early changes in inositol lipids and their metabolites induced by platelet-derived growth factor in quiescent Swiss mouse 3T3 cells. *Biochem J*, **232**: 99 - 109.
Hernandez-Torres G, Cipriano M, Heden E, Bjorklund E, Canales A, Zian D, Feliu A, Mecha M, Guaza C, Fowler CJ, Ortega-Gutierrez S, Lopez-Rodriguez ML (2014). A reversible and selective inhibitor of monoacylglycerol lipase ameliorates multiple sclerosis. *Angew Chem Int Ed Engl*, **53**: 13765 - 13770.
Hill MN, Bialecki J, Wellinger NL, Thompson RJ (2015). Pannexin-1 channels permit post-synaptic anandamide transport in hippocampal pyramidal neurons. *25th annual symposium of the International Cannabinoid Research Society*,34 -
Hoover HS, Blankman JL, Niessen S, Cravatt BF (2008). Selectivity of inhibitors of endocannabinoid biosynthesis evaluated by activity-based protein profiling. *Bioorg Med Chem Lett*, **18**: 5838 - 5841.
Hsu KL, Tsuboi K, Adibekian A, Pugh H, Masuda K, Cravatt BF (2012). DAGLβ inhibition perturbs a lipid network involved in macrophage inflammatory responses. *Nat Chem Biol*, **8**: 999 - 1007.
Hsu KL, Tsuboi K, Chang JW, Whitby LR, Speers AE, Pugh H, Cravatt BF (2013). Discovery and Optimization of Piperidyl-1,2,3-Triazole Ureas as Potent, Selective, and in Vivo-Active Inhibitors of α/β-Hydrolase Domain Containing 6 (ABHD6). *J Med Chem*, **56**: 8270 - 8279.
Huggins JP, Smart TS, Langman S, Taylor L, Young T (2012). An efficient randomised, placebo-controlled clinical trial with the irreversible fatty acid amide hydrolase-1 inhibitor PF-04457845, which modulates endocannabinoids but fails to induce effective analgesia in patients with pain due to osteoarthritis of the knee. *Pain*, **153**: 1837 - 1846.
Janssen FJ, Baggelaar MP, Hummel JJ, Overkleeft HS, Cravatt BF, Boger DL, van der Stelt M (2015). Comprehensive Analysis of Structure Activity Relationships of α-Ketoheterocycles as *sn*-1-Diacylglycerol Lipase alpha Inhibitors. *J Med Chem*, **58**: 9742 - 9753.
Janssen FJ, van der Stelt M (2016). Inhibitors of diacylglycerol lipases in neurodegenerative and metabolic disorders. *Bioorg Med Chem Lett*, **26**: 3831 - 3837.
Jarai Z, Wagner JA, Goparaju SK, Wang L, Razdan RK, Sugiura T, Zimmer AM, Bonner TI, Zimmer A, Kunos G (2000). Cardiovascular effects of 2-arachidonoyl glycerol in anesthetized mice. *Hypertension*, **35**: 679 - 684.
Jenniches I, Ternes S, Albayram O, Otte DM, Bach K, Bindila L, Michel K, Lutz B, Bilkei-Gorzo A, Zimmer A (2016). Anxiety, Stress, and Fear Response in Mice with Reduced Endocannabinoid Levels. *Biol Psychiatry*, **79**: 858 - 868.
Jhaveri MD, Richardson D, Kendall DA, Barrett DA, Chapman V (2006). Analgesic effects of fatty acid amide hydrolase inhibition in a rat model of neuropathic pain. *J Neurosci*, **26**: 13318 - 13327.
Jin S, Zhao G, Li Z, Nishimoto Y, Isohama Y, Shen J, Ito T, Takeya M, Araki K, He P, Yamamura K (2009). Age-related pulmonary emphysema in mice lacking α/β hydrolase domain containing 2 gene. *Biochem Biophys Res Commun*, **380**: 419 - 424.

Jung KM, Sepers M, Henstridge CM, Lassalle O, Neuhofer D, Martin H, Ginger M, Frick A, DiPatrizio NV, Mackie K, Katona I, Piomelli D, Manzoni OJ (2012). Uncoupling of the endocannabinoid signalling complex in a mouse model of fragile X syndrome. *Nat Commun*, **3**: 1080 -
Kaczocha M, Glaser ST, Chae J, Brown DA, Deutsch DG (2010). Lipid droplets are novel sites of *N*-acylethanolamine inactivation by fatty acid amide hydrolase-2. *J Biol Chem*, **285**: 2795 - 2806.
Kaczocha M, Glaser ST, Deutsch DG (2009). Identification of intracellular carriers for the endocannabinoid anandamide. *Proc Natl Acad Sci U S A*, **106**: 6375 - 6380.
Kaczocha M, Glaser ST, Maher T, Clavin B, Hamilton J, O'Rourke J, Rebecchi M, Puopolo M, Owada Y, Thanos PK (2015). Fatty acid binding protein deletion suppresses inflammatory pain through endocannabinoid/*N*-acylethanolamine-dependent mechanisms. *Mol Pain*, **11**: 52 -
Kamat SS, Camara K, Parsons WH, Chen DH, Dix MM, Bird TD, Howell AR, Cravatt BF (2015). Immunomodulatory lysophosphatidylserines are regulated by ABHD16A and ABHD12 interplay. *Nat Chem Biol*, **11**: 164 - 171.
Karbarz MJ, Luo L, Chang L, Tham CS, Palmer JA, Wilson SJ, Wennerholm ML, Brown SM, Scott BP, Apodaca RL, Keith JM, Wu J, Breitenbucher JG, Chaplan SR, Webb M (2009). Biochemical and biological properties of 4-(3-phenyl-[1,2,4] thiadiazol-5-yl)-piperazine-1-carboxylic acid phenylamide, a mechanism-based inhibitor of fatty acid amide hydrolase. *Anesth Analg*, **108**: 316 - 329.
Karlsson J, Morgillo CM, Deplano A, Smaldone G, Pedone E, Luque FJ, Svensson M, Novellino E, Congiu C, Onnis V, Catalanotti B, Fowler CJ (2015). Interaction of the N-(3-Methylpyridin-2-yl) amide Derivatives of Flurbiprofen and Ibuprofen with FAAH: Enantiomeric Selectivity and Binding Mode. *PLoS One*, **10**:
Kathuria S, Gaetani S, Fegley D, Valino F, Duranti A, Tontini A, Mor M, Tarzia G, Rana GL, Calignano A, Giustino A, Tattoli M, Palmery M, Cuomo V, Piomelli D (2003). Modulation of anxiety through blockade of anandamide hydrolysis. *Nat Med*, **9**: 76 - 81.
Katona I, Urban GM, Wallace M, Ledent C, Jung KM, Piomelli D, Mackie K, Freund TF (2006). Molecular composition of the endocannabinoid system at glutamatergic synapses. *J Neurosci*, **26**: 5628 - 5637.
Kerr DM, Downey L, Conboy M, Finn DP, Roche M (2013). Alterations in the endocannabinoid system in the rat valproic acid model of autism. *Behav Brain Res*, **249**: 124 - 132.
Kohnz RA, Nomura DK (2014). Chemical approaches to therapeutically target the metabolism and signaling of the endocannabinoid 2-AG and eicosanoids. *Chem Soc Rev*, **43**: 6859 - 6869.
Kozak KR, Gupta RA, Moody JS, Ji C, Boeglin WE, DuBois RN, Brash AR, Marnett LJ (2002). 15-lipoxygenase metabolism of 2-arachidonylglycerol: Generation of a peroxisome proliferator-activated receptor α agonist. *J Biol Chem*, **277**: 23278 - 23286.
Kozak KR, Rowlinson SW, Marnett LJ (2000). Oxygenation of the endocannabinoid, 2-arachidonylglycerol, to glyceryl prostaglandins by cyclooxygenase-2. *J Biol Chem*, **275**: 33744 - 33749.
Kuwae T, Shiota Y, Schmid PC, Krebsbach R, Schmid HHO (1999). Biosynthesis and turnover of anandamide and other N-acylethanolamines in peritoneal macrophages. *FEBS Lett*, **459**: 123 - 127.
Lee SH, Ledri M, Toth B, Marchionni I, Henstridge CM, Dudok B, Kenesei K, Barna L, Szabo SI, Renkecz T, Oberoi M, Watanabe M, Limoli CL, Horvai G, Soltesz I, Katona I (2015). Multiple Forms of Endocannabinoid and Endovanilloid Signaling Regulate the Tonic Control of GABA Release. *J Neurosci*, **35**: 10039 - 10057.
Leishman E, Mackie K, Luquet S, Bradshaw HB (2016). Lipidomics profile of a NAPE-PLD KO mouse provides evidence of a broader role of this enzyme in lipid metabolism in the brain. *Biochim Biophys Acta*, **1861**: 491 - 500.
Leung D, Saghatelian A, Simon GM, Cravatt BF (2006). Inactivation of *N*-acyl phosphatidylethanolamine phospholipase D reveals multiple mechanisms for the biosynthesis of endocannabinoids. *Biochemistry*, **45**: 4720 - 4726.
Leung K, Elmes MW, Glaser ST, Deutsch DG, Kaczocha M (2013). Role of FAAH-like anandamide transporter in anandamide inactivation. *PLoS One*, **8**: e79355 -

Li W, Blankman JL, Cravatt BF (2007). A functional proteomic strategy to discover inhibitors for uncharacterized hydrolases. *J Am Chem Soc*, **129**: 9594 - 9595.
Liu J, Batkai S, Pacher P, Harvey-White J, Wagner JA, Cravatt BF, Gao B, Kunos G (2003). Lipopolysaccharide induces anandamide synthesis in macrophages via CD14/MAPK/phosphoinositide 3-kinase/NF-κB independently of platelet activating factor. *J Biol Chem*, **278**: 45034 - 45039.
Liu J, Wang L, Harvey-White J, Osei-Hyiaman D, Razdan R, Gong Q, Chan AC, Zhou ZF, Huang BX, Kim HY, Kunos G (2006). A biosynthetic pathway for anandamide. *Proc Natl Acad Sci U S A*, **103**: 13345 - 13350.
Long JZ, Li W, Booker L, Burston JJ, Kinsey SG, Schlosburg JE, Pavon FJ, Serrano AM, Selley DE, Parsons LH, Lichtman AH, Cravatt BF (2009). Selective blockade of 2-arachidonoylglycerol hydrolysis produces cannabinoid behavioral effects. *Nat Chem Biol*, **5**: 37 - 44.
Maccarrone M, De Petrocellis L, Bari M, Fezza F, Salvati S, Di Marzo V, Finazzi-Agro A (2001a). Lipopolysaccharide downregulates fatty acid amide hydrolase expression and increases anandamide levels in human peripheral lymphocytes. *Arch Biochem Biophys*, **393**: 321 - 328.
Maccarrone M, Guzman M, Mackie K, Doherty P, Harkany T (2014). Programming of neural cells by (endo)cannabinoids: from physiological rules to emerging therapies. *Nat Rev Neurosci*, **15**: 786 - 801.
Maccarrone M, Valensise H, Bari M, Lazzarin N, Romanini C, Finazzi-Agro A (2001b). Progesterone up-regulates anandamide hydrolase in human lymphocytes: role of cytokines and implications for fertility. *J Immunol*, **166**: 7183 - 7189.
Manna JD, Wepy JA, Hsu K, Chang JW, Cravatt BF, Marnett LJ (2014). Identification of the Major Prostaglandin Glycerol Ester Hydrolase in Human Cancer Cells. *J Biol Chem*, **289**: 33741 - 33753.
Margheritis E, Castellani B, Magotti P, Peruzzi S, Romeo E, Natali F, Mostarda S, Gioiello A, Piomelli D, Garau G (2016). Bile Acid Recognition by NAPE-PLD. *ACS Chem Biol*, **11**: 2908 - 2914.
McDougle DR, Kambalyal A, Meling DD, Das A (2014). Endocannabinoids anandamide and 2-arachidonoylglycerol are substrates for human CYP2J2 epoxygenase. *J Pharmacol Exp Ther*, **351**: 616 - 627.
McKinney MK, Cravatt BF (2006). Structure-based design of a FAAH variant that discriminates between the *N*-acyl ethanolamine and taurine families of signaling lipids. *Biochemistry*, **45**: 9016 - 9022.
Mechoulam R, Benshabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR, Gopher A, Almog S, Martin BR, Compton DR, Pertwee RG, Griffin G, Bayewitch M, Barg J, Vogel Z (1995). Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem Pharmacol*, **50**: 83 - 90.
Migliore M, Pontis S, Fuentes de Arriba AL, Realini N, Torrente E, Armirotti A, Romeo E, Di Martino S, Russo D, Pizzirani D, Summa M, Lanfranco M, Ottonello G, Busquet P, Jung KM, Garcia-Guzman M, Heim R, Scarpelli R, Piomelli D (2016). Second-Generation Non-Covalent NAAA Inhibitors are Protective in a Model of Multiple Sclerosis. *Angew Chem Int Ed Engl*, **55**: 11193 - 11197.
Mileni M, Johnson DS, Wang Z, Everdeen DS, Liimatta M, Pabst B, Bhattacharya K, Nugent RA, Kamtekar S, Cravatt BF, Ahn K, Stevens RC (2008). Structure-guided inhibitor design for human FAAH by interspecies active site conversion. *Proc Natl Acad Sci U S A*, **105**: 12820 - 12824.
Miller MR, Mannowetz N, Iavarone AT, Safavi R, Gracheva EO, Smith JF, Hill RZ, Bautista DM, Kirichok Y, Lishko PV (2016). Unconventional endocannabinoid signaling governs sperm activation via the sex hormone progesterone. *Science*, **352**: 555 - 559.
Mitchener MM, Hermanson DJ, Shockley EM, Brown HA, Lindsley CW, Reese J, Rouzer CA, Lopez CF, Marnett LJ (2015). Competition and allostery govern substrate selectivity of cyclooxygenase-2. *Proc Natl Acad Sci U S A*, **112**: 12366 - 12371.
Miyata K, Nakayama M, Mizuta S, Hokimoto S, Sugamura K, Oshima S, Oike Y, Sugiyama S, Ogawa H, Yamamura K (2008). Elevated mature macrophage expression of human ABHD2 gene in vulnerable plaque. *Biochem Biophys Res Commun*, **365**: 207 - 213.

Miyata K, Oike Y, Hoshii T, Maekawa H, Ogawa H, Suda T, Araki K, Yamamura K (2005). Increase of smooth muscle cell migration and of intimal hyperplasia in mice lacking the α/β hydrolase domain containing 2 gene. *Biochem Biophys Res Commun*, **329**: 296 - 304.
Moody JS, Kozak KR, Ji C, Marnett LJ (2001). Selective oxygenation of the endocannabinoid 2-arachidonylglycerol by leukocyte-type 12-lipoxygenase. *Biochemistry*, **40**: 861 - 866.
Moreira FA, Crippa JA (2009). The psychiatric side-effects of rimonabant. *Rev Bras Psiquiatr*, **31**: 145 - 153.
Moriuchi H, Koda N, Okuda-Ashitaka E, Daiyasu H, Ogasawara K, Toh H, Ito S, Woodward DF, Watanabe K (2008). Molecular characterization of a novel type of prostamide/prostaglandin F synthase, belonging to the thioredoxin-like superfamily. *J Biol Chem*, **283**: 792 - 801.
Mulder AM, Cravatt BF (2006). Endocannabinoid metabolism in the absence of fatty acid amide hydrolase (FAAH): discovery of phosphorylcholine derivatives of *N*-acyl ethanolamines. *Biochemistry*, **45**: 11267 - 11277.
Naresh Kumar M, Thunuguntla VB, Veeramachaneni GK, Chandra Sekhar B, Guntupalli S, Bondili JS (2016). Molecular characterization of human ABHD2 as TAG lipase and ester hydrolase. *Biosci Rep*, **36**:
Natarajan V, Reddy PV, Schmid PC, Schmid HHO (1982). *N*-Acylation of ethanolamine phospholipids in canine myocardium. *Biochim Biophys Acta*, **712**: 342 - 355.
Natarajan V, Schmid PC, Reddy PV, Zuzarte-Augustin ML, Schmid HH (1983). Biosynthesis of N-acylethanolamine phospholipids by dog brain preparations. *J Neurochem*, **41**: 1303 - 1312.
Navia-Paldanius D, Savinainen JR, Laitinen JT (2012). Biochemical and pharmacological characterization of human α/β-hydrolase domain containing 6 (ABHD6) and 12 (ABHD12). *J Lipid Res*, **53**: 2413 - 2424.
Naydenov AV, Horne EA, Cheah CS, Swinney K, Hsu KL, Cao JK, Marrs WR, Blankman JL, Tu S, Cherry AE, Fung S, Wen A, Li W, Saporito MS, Selley DE, Cravatt BF, Oakley JC, Stella N (2014). ABHD6 Blockade Exerts Antiepileptic Activity in PTZ-Induced Seizures and in Spontaneous Seizures in R6/2 Mice. *Neuron*, **83**: 361 - 371.
Nicolussi S, Gertsch J (2015). Endocannabinoid transport revisited. *Vitam Horm*, **98**: 441 - 485.
Nomura DK, Morrison BE, Blankman JL, Long JZ, Kinsey SG, Marcondes MC, Ward AM, Hahn YK, Lichtman AH, Conti B, Cravatt BF (2011). Endocannabinoid hydrolysis generates brain prostaglandins that promote neuroinflammation. *Science*, **334**: 809 - 813.
Nyilas R, Dudok B, Urban GM, Mackie K, Watanabe M, Cravatt BF, Freund TF, Katona I (2008). Enzymatic machinery for endocannabinoid biosynthesis associated with calcium stores in glutamatergic axon terminals. *J Neurosci*, **28**: 1058 - 1063.
Obinata D, Takada S, Takayama KI, Urano T, Ito A, Ashikari D, Fujiwara K, Yamada Y, Murata T, Kumagai J, Fujimura T, Ikeda K, Horie-Inoue K, Homma Y, Takahashi S, Inoue S (2016). Abhydrolase domain containing 2, an androgen target gene, promotes prostate cancer cell proliferation and migration. *Eur J Cancer*, **57**: 39 - 49.
Oddi S, Fezza F, Pasquariello N, D'Agostino A, Catanzaro G, De Simone C, Rapino C, Finazzi-Agro A, Maccarrone M (2009). Molecular identification of albumin and Hsp70 as cytosolic anandamide-binding proteins. *Chem Biol*, **16**: 624 - 632.
Ogasawara D, Deng H, Viader A, Baggelaar MP, Breman A, den Dulk H, van den Nieuwendijk AM, Soethoudt M, van der Wel T, Zhou J, Overkleeft HS, Sanchez-Alavez M, Mo S, Nguyen W, Conti B, Liu X, Chen Y, Liu QS, Cravatt BF, van der Stelt M (2016). Rapid and profound rewiring of brain lipid signaling networks by acute diacylglycerol lipase inhibition. *Proc Natl Acad Sci U S A*, **113**: 26 - 33.
Ogura Y, Parsons WH, Kamat SS, Cravatt BF (2016). A calcium-dependent acyltransferase that produces *N*-acyl phosphatidylethanolamines. *Nat Chem Biol*, **12**: 669 - 671.
Ohno-Shosaku T, Kano M (2014). Endocannabinoid-mediated retrograde modulation of synaptic transmission. *Curr Opin Neurobiol*, **29C**: 1 - 8.
Okamoto Y, Morishita J, Tsuboi K, Tonai T, Ueda N (2004a). Molecular characterization of a phospholipase D generating anandamide and its congeners. *J Biol Chem*, **279**: 5298 - 5305.

Okamoto Y, Morishita J, Tsuboi K, Tonai T, Ueda N (2004b). Molecular characterization of a phospholipase D generating anandamide and its congeners. *J Biol Chem*, **279**: 5298 - 5305.
Oudin MJ, Hobbs C, Doherty P (2011). DAGL-dependent endocannabinoid signalling: roles in axonal pathfinding, synaptic plasticity and adult neurogenesis. *Eur J Neurosci*, **34**: 1634 - 1646.
Palermo G, Bauer I, Campomanes P, Cavalli A, Armirotti A, Girotto S, Rothlisberger U, De Vivo M (2015). Keys to Lipid Selection in Fatty Acid Amide Hydrolase Catalysis: Structural Flexibility, Gating Residues and Multiple Binding Pockets. *PLoS Comput Biol*, **11**: e1004231 -
Pan B, Wang W, Zhong P, Blankman JL, Cravatt BF, Liu QS (2011). Alterations of Endocannabinoid Signaling, Synaptic Plasticity, Learning, and Memory in Monoacylglycerol Lipase Knock-out Mice. *J Neurosci*, **31**: 13420 - 13430.
Paria BC, Wang H, Dey SK (2002). Endocannabinoid signaling in synchronizing embryo development and uterine receptivity for implantation. *Chem Phys Lipids*, **121**: 201 - 210.
Piro JR, Benjamin DI, Duerr JM, Pi Y, Gonzales C, Wood KM, Schwartz JW, Nomura DK, Samad TA (2012). A dysregulated endocannabinoid-eicosanoid network supports pathogenesis in a mouse model of Alzheimer's disease. *Cell Rep*, **1**: 617 - 623.
Powell DR, Gay JP, Wilganowski N, Doree D, Savelieva KV, Lanthorn TH, Read R, Vogel P, Hansen GM, Brommage R, Ding ZM, Desai U, Zambrowicz B (2015). Diacylglycerol Lipase α Knockout Mice Demonstrate Metabolic and Behavioral Phenotypes Similar to Those of Cannabinoid Receptor 1 Knockout Mice. *Front Endocrinol (Lausanne)*, **6**: 86 -
Pratt-Hyatt M, Zhang H, Snider NT, Hollenberg PF (2010). Effects of a commonly occurring genetic polymorphism of human CYP3A4 (I118V) on the metabolism of anandamide. *Drug Metab Dispos*, **38**: 2075 - 2082.
Prescott SM, Majerus PW (1983). Characterization of 1,2-diacylglycerol hydrolysis in human platelets. Demonstration of an arachidonoyl-monoacylglycerol intermediate. *J Biol Chem*, **258**: 764 - 769.
Pribasnig MA, Mrak I, Grabner GF, Taschler U, Knittelfelder O, Scherz B, Eichmann TO, Heier C, Grumet L, Kowaliuk J, Romauch M, Holler S, Anderl F, Wolinski H, Lass A, Breinbauer R, Marsche G, Brown JM, Zimmermann R (2015). α/β Hydrolase Domain-Containing 6 (ABHD6) Degrades the Late Endosomal/Lysosomal Lipid Bis(monoacylglycero)phosphate. *J Biol Chem*, **290**: 29869 - 29881.
Rahman IA, Tsuboi K, Hussain Z, Yamashita R, Okamoto Y, Uyama T, Yamazaki N, Tanaka T, Tokumura A, Ueda N (2016). Calcium-dependent generation of N-acylethanolamines and lysophosphatidic acids by glycerophosphodiesterase GDE7. *Biochim Biophys Acta*, **1861**: 1881 - 1892.
Ramikie TS, Nyilas R, Bluett RJ, Gamble-George JC, Hartley ND, Mackie K, Watanabe M, Katona I, Patel S (2014). Multiple mechanistically distinct modes of endocannabinoid mobilization at central amygdala glutamatergic synapses. *Neuron*, **81**: 1111 - 1125.
Reisenberg M, Singh PK, Williams G, Doherty P (2012). The diacylglycerol lipases: structure, regulation and roles in and beyond endocannabinoid signalling. *Philos Trans R Soc Lond B Biol Sci*, **367**: 3264 - 3275.
Ribeiro A, Pontis S, Mengatto L, Armirotti A, Chiurchiu V, Capurro V, Fiasella A, Nuzzi A, Romeo E, Moreno-Sanz G, Maccarrone M, Reggiani A, Tarzia G, Mor M, Bertozzi F, Bandiera T, Piomelli D (2015). A Potent Systemically Active N-Acylethanolamine Acid Amidase Inhibitor that Suppresses Inflammation and Human Macrophage Activation. *ACS Chem Biol*, **10**: 1838 - 1846.
Rosenberger TA, Farooqui AA, Horrocks LA (2007). Bovine brain diacylglycerol lipase: substrate specificity and activation by cyclic AMP-dependent protein kinase. *Lipids*, **42**: 187 - 195.
Sanson B, Wang T, Sun J, Wang L, Kaczocha M, Ojima I, Deutsch D, Li H (2014). Crystallographic study of FABP5 as an intracellular endocannabinoid transporter. *Acta Crystallogr D Biol Crystallogr*, **70**: 290 - 298.
Sasso O, Migliore M, Habrant D, Armirotti A, Albani C, Summa M, Moreno-Sanz G, Scarpelli R, Piomelli D (2015). Multitarget fatty acid amide hydrolase/cyclooxygenase blockade suppresses intestinal inflammation and protects against nonsteroidal anti-inflammatory drug-dependent gastrointestinal damage. *FASEB J*, **29**: 2616 - 2627.

Schmid PC, Krebsbach RJ, Perry SR, Dettmer TM, Maasson JL, Schmid HHO (1995). Occurrence and postmortem generation of anandamide and other long chain *N*-acylethanolamines in mammalian brain. *FEBS Lett*, **375**: 117 - 120.
Schmid PC, Reddy PV, Natarajan V, Schmid HHO (1983). Metabolism of N-acylethanolamine phospholipids by a mammalian phosphodiesterase of the phospholipase D type. *J Biol Chem*, **258**: 9302 - 9306.
Sciolino NR, Zhou W, Hohmann AG (2011). Enhancement of endocannabinoid signaling with JZL184, an inhibitor of the 2-arachidonoylglycerol hydrolyzing enzyme monoacylglycerol lipase, produces anxiolytic effects under conditions of high environmental aversiveness in rats. *Pharmacol Res*, **64**: 226 - 234.
Shonesy BC, Bluett RJ, Ramikie TS, Baldi R, Hermanson DJ, Kingsley PJ, Marnett LJ, Winder DG, Colbran RJ, Patel S (2014). Genetic disruption of 2-arachidonoylglycerol synthesis reveals a key role for endocannabinoid signaling in anxiety modulation. *Cell Rep*, **9**: 1644 - 1653.
Singh PK, Markwick R, Lu L, Howell FV, Williams G, Doherty P (2016). Assay and Inhibition of the Purified Catalytic Domain of Diacylglycerol Lipase Beta. *Biochemistry*, **55**: 2713 - 2721.
Snider NT, Kornilov AM, Kent UM, Hollenberg PF (2007). Anandamide metabolism by human liver and kidney microsomal cytochrome P450 enzymes to form hydroxyeicosatetraenoic and epoxyeicosatrienoic acid ethanolamides. *J Pharmacol Exp Ther*, **321**: 590 - 597.
Snider NT, Nast JA, Tesmer LA, Hollenberg PF (2009). A cytochrome P450-derived epoxygenated metabolite of anandamide is a potent cannabinoid receptor 2-selective agonist. *Mol Pharmacol*, **75**: 965 - 972.
Snider NT, Sikora MJ, Sridar C, Feuerstein TJ, Rae JM, Hollenberg PF (2008). The endocannabinoid anandamide is a substrate for the human polymorphic cytochrome P450 2D6. *J Pharmacol Exp Ther*, **327**: 538 - 545.
Solorzano C, Zhu C, Battista N, Astarita G, Lodola A, Rivara S, Mor M, Russo R, Maccarrone M, Antonietti F, Duranti A, Tontini A, Cuzzocrea S, Tarzia G, Piomelli D (2009). Selective *N*-acylethanolamine-hydrolyzing acid amidase inhibition reveals a key role for endogenous palmitoylethanolamide in inflammation. *Proc Natl Acad Sci U S A*, **106**: 20966 - 20971.
Sridar C, Snider NT, Hollenberg PF (2011). Anandamide oxidation by wild-type and polymorphically expressed CYP2B6 and CYP2D6. *Drug Metab Dispos*, **39**: 782 - 788.
Sticht MA, Long JZ, Rock EM, Limebeer CL, Mechoulam R, Cravatt BF, Parker LA (2012). Inhibition of monoacylglycerol lipase attenuates vomiting in *Suncus murinus* and 2-arachidonoyl glycerol attenuates nausea in rats. *Br J Pharmacol*, **165**: 2425 - 2435.
Sugiura T, Kondo S, Sukagawa A, Nakane S, Shinoda A, Itoh K, Yamashita A, Waku K (1995). 2-Arachidonoylgylcerol: a possible endogenous cannabinoid receptor ligand in brain. *Biochem Biophys Res Commun*, **215**: 89 - 97.
Sugiura T, Waku K (2000). 2-Arachidonoylglycerol and the cannabinoid receptors. *Chem Phys Lipids*, **108**: 89 - 106.
Sun YX, Tsuboi K, Okamoto Y, Tonai T, Murakami M, Kudo I, Ueda N (2004). Biosynthesis of anandamide and *N*-palmitoylethanolamine by sequential actions of phospholipase A_2 and lysophospholipase D. *Biochem J*, **380**: 749 - 756.
Tai T, Tsuboi K, Uyama T, Masuda K, Cravatt BF, Houchi H, Ueda N (2012). Endogenous Molecules Stimulating N-Acylethanolamine-Hydrolyzing Acid Amidase (NAAA). *ACS Chem Neurosci*, **3**: 379 - 385.
Tanimura A, Yamazaki M, Hashimotodani Y, Uchigashima M, Kawata S, Abe M, Kita Y, Hashimoto K, Shimizu T, Watanabe M, Sakimura K, Kano M (2010). The Endocannabinoid 2-Arachidonoylglycerol Produced by Diacylglycerol Lipase alpha Mediates Retrograde Suppression of Synaptic Transmission. *Neuron*, **65**: 320 - 327.
Tchantchou F, Zhang Y (2013). Selective inhibition of alpha/beta-hydrolase domain 6 attenuates neurodegeneration, alleviates blood brain barrier breakdown and improves functional recovery in a mouse model of traumatic brain injury. *J Neurotrauma*, **30**: 565 - 579.

Thomas G, Betters JL, Lord CC, Brown AL, Marshall S, Ferguson D, Sawyer J, Davis MA, Melchior JT, Blume LC, Howlett AC, Ivanova PT, Milne SB, Myers DS, Mrak I, Leber V, Heier C, Taschler U, Blankman JL, Cravatt BF, Lee RG, Crooke RM, Graham MJ, Zimmermann R, Brown HA, Brown JM (2013). The serine hydrolase ABHD6 Is a critical regulator of the metabolic syndrome. *Cell Rep*, **5**: 508 - 520.
Thomas G, Brown AL, Brown JM (2014). *In vivo* metabolite profiling as a means to identify uncharacterized lipase function: Recent success stories within the alpha beta hydrolase domain (ABHD) enzyme family. *Biochim Biophys Acta*, **1841**: 1097 - 1101.
Thors L, Bergh A, Persson E, Hammarsten P, Stattin P, Egevad L, Granfors T, Fowler CJ (2010a). Fatty acid amide hydrolase in prostate cancer: association with disease severity and outcome, CB1 receptor expression and regulation by IL-4. *PLoS One*, **5**: e12275 -
Thors L, Burston JJ, Alter BJ, McKinney MK, Cravatt BF, Ross RA, Pertwee RG, Gereau RW, Wiley JL, Fowler CJ (2010b). Biochanin A, a naturally occurring inhibitor of fatty acid amide hydrolase. *Br J Pharmacol*, **160**: 549 - 560.
Tortoriello G, Rhodes BP, Takacs SM, Stuart JM, Basnet A, Raboune S, Widlanski TS, Doherty P, Harkany T, Bradshaw HB (2013). Targeted Lipidomics in Drosophila melanogaster Identifies Novel 2-Monoacylglycerols and N-acyl Amides. *PLoS One*, **8**: e67865 -
Tsuboi K, Okamoto Y, Ikematsu N, Inoue M, Shimizu Y, Uyama T, Wang J, Deutsch DG, Burns MP, Ulloa NM, Tokumura A, Ueda N (2011). Enzymatic formation of N-acylethanolamines from N-acylethanolamine plasmalogen through N-acylphosphatidylethanolamine-hydrolyzing phospholipase D-dependent and -independent pathways. *Biochim Biophys Acta*, **1811**: 565 - 577.
Tsuboi K, Sun YX, Okamoto Y, Araki N, Tonai T, Ueda N (2005). Molecular characterization of N-acylethanolamine-hydrolyzing acid amidase, a novel member of the choloylglycine hydrolase family with structural and functional similarity to acid ceramidase. *J Biol Chem*, **280**: 11082 - 11092.
Tsuboi K, Zhao LY, Okamoto Y, Araki N, Ueno M, Sakamoto H, Ueda N (2007). Predominant expression of lysosomal N-acylethanolamine-hydrolyzing acid amidase in macrophages revealed by immunochemical studies. *Biochim Biophys Acta-Mol Cell Biol Lip*, **1771**: 623 - 632.
Uchigashima M, Narushima M, Fukaya M, Katona I, Kano M, Watanabe M (2007). Subcellular arrangement of molecules for 2-arachidonoyl-glycerol-mediated retrograde signaling and its physiological contribution to synaptic modulation in the striatum. *J Neurosci*, **27**: 3663 - 3676.
Ueda N, Tsuboi K, Uyama T (2013). Metabolism of endocannabinoids and related N-acylethanolamines: canonical and alternative pathways. *FEBS J*, **280**: 1874 - 1894.
Ueda N, Yamamoto K, Yamamoto S, Tokunaga T, Shirakawa E, Shinkai H, Ogawa M, Sato T, Kudo I, Inoue K, Takizawa H, Nagano T, Hirobe M, Matsuki N, Saito H (1995). Lipoxygenase-catalyzed oxygenation of arachidonylethanolamide, a cannabinoid receptor agonist. *Biochim Biophys Acta-Lip Lip Met*, **1254**: 127 - 134.
Ueda N, Yamanaka K, Yamamoto S (2001). Purification and characterization of an acid amidase selective for *N*-palmitoylethanolamine, a putative endogenous anti-inflammatory substance. *J Biol Chem*, **276**: 35552 - 35557.
Uyama T, Ikematsu N, Inoue M, Shinohara N, Jin XH, Tsuboi K, Tonai T, Tokumura A, Ueda N (2012). Generation of N-Acylphosphatidylethanolamine by Members of the Phospholipase A/Acyltransferase (PLA/AT) Family. *J Biol Chem*, **287**: 31905 - 31919.
van der Stelt M, van Kuik JA, Bari M, Van Zadelhoff G, Leeflang BR, Veldink GA, Finazzi-Agro A, Vliegenthart JF, Maccarrone M (2002). Oxygenated metabolites of anandamide and 2-arachidonoylglycerol: conformational analysis and interaction with cannabinoid receptors, membrane transporter, and Fatty Acid amide hydrolase. *J Med Chem*, **45**: 3709 - 3720.
van Tienhoven M, Atkins J, Li Y, Glynn P (2002). Human neuropathy target esterase catalyzes hydrolysis of membrane lipids. *J Biol Chem*, **277**: 20942 - 20948.
Vandevoorde S, Saha B, Mahadevan A, Razdan RK, Pertwee RG, Martin BR, Fowler CJ (2005). Influence of the degree of unsaturation of the acyl side chain upon the interaction of analogues of 1-

arachidonoylglycerol with monoacylglycerol lipase and fatty acid amide hydrolase. *Biochem Biophys Res Commun*, **337**: 104 - 109.
Vaughan M, BERGER JE, Steinberg D (1964). Hormone-sensitive lipase and monoglyceride lipase activities in adipose tissue. *J Biol Chem*, **239**: 401 - 409.
Vecchio AJ, Malkowski MG (2011). The structural basis of endocannabinoid oxygenation by cyclooxygenase-2. *J Biol Chem*, **286**: 20736 - 20745.
Viader A, Ogasawara D, Joslyn CM, Sanchez-Alavez M, Mori S, Nguyen W, Conti B, Cravatt BF (2016). A chemical proteomic atlas of brain serine hydrolases identifies cell type-specific pathways regulating neuroinflammation. *Elife*, **5**:
Walker VJ, Griffin AP, Hammar DK, Hollenberg PF (2016). Metabolism of Anandamide by Human Cytochrome P450 2J2 in the Reconstituted System and Human Intestinal Microsomes. *J Pharmacol Exp Ther*, **357**: 537 - 544.
Wei BQ, Mikkelsen TS, McKinney MK, Lander ES, Cravatt BF (2006). A second fatty acid amide hydrolase with variable distribution among placental mammals. *J Biol Chem*, **281**: 36569 - 36578.
Wen J, Ribeiro R, Tanaka M, Zhang Y (2015). Activation of CB2 receptor is required for the therapeutic effect of ABHD6 inhibition in experimental autoimmune encephalomyelitis. *Neuropharmacology*, **99**: 196 - 209.
Woodhams SG, Wong A, Barrett DA, Bennett AJ, Chapman V, Alexander SPH (2012). Spinal administration of the monoacylglycerol lipase inhibitor JZL184 produces robust inhibitory effects on nociceptive processing and the development of central sensitization in the rat. *Br J Pharmacol*, **167**: 1609 - 1619.
Woodward DF, Wang JW, Poloso NJ (2013). Recent progress in prostaglandin f2alpha ethanolamide (prostamide f2alpha) research and therapeutics. *Pharmacol Rev*, **65**: 1135 - 1147.
Xie SQ, Borazjani A, Hatfield MJ, Edwards CC, Potter PM, Ross MK (2010). Inactivation of lipid glyceryl ester metabolism in human THP1 monocytes/macrophages by activated organophosphorus insecticides: role of carboxylesterases 1 and 2. *Chemical Research in Toxicology*, **23**: 1890 - 1904.
Yamanoi K, Matsumura N, Murphy SK, Baba T, Abiko K, Hamanishi J, Yamaguchi K, Koshiyama M, Konishi I, Mandai M (2016). Suppression of ABHD2, identified through a functional genomics screen, causes anoikis resistance, chemoresistance and poor prognosis in ovarian cancer. *Oncotarget*, **7**: 47620 - 47636.
Yang W, Ni JS, Woodward DF, Tang-Liu DDS, Ling KHJ (2005). Enzymatic formation of prostamide $F_{2\alpha}$ from anandamide involves a newly identified intermediate metabolite, prostamide H_2. *J Lipid Res*, **46**: 2745 - 2751.
Yoshida T, Fukaya M, Uchigashima M, Miura E, Kamiya H, Kano M, Watanabe M (2006). Localization of diacylglycerol lipase-α around postsynaptic spine suggests close proximity between production site of an endocannabinoid, 2-arachidonoyl-glycerol, and presynaptic cannabinoid CB1 receptor. *J Neurosci*, **26**: 4740 - 4751.
Yoshida T, Uchigashima M, Yamasaki M, Katona I, Yamazaki M, Sakimura K, Kano M, Yoshioka M, Watanabe M (2011). Unique inhibitory synapse with particularly rich endocannabinoid signaling machinery on pyramidal neurons in basal amygdaloid nucleus. *Proc Natl Acad Sci U S A*, **108**: 3059 - 3064.
Yoshino H, Miyamae T, Hansen G, Zambrowicz B, Flynn M, Pedicord D, Blat Y, Westphal RS, Zaczek R, Lewis DA, Gonzalez-Burgos G (2011). Postsynaptic diacylglycerol lipase mediates retrograde endocannabinoid suppression of inhibition in mouse prefrontal cortex. *J Physiol*, **589**: 4857 - 4884.
Younts TJ, Castillo PE (2014). Endogenous cannabinoid signaling at inhibitory interneurons. *Curr Opin Neurobiol*, **26**: 42 - 50.
Yu M, Ives D, Ramesha CS (1997). Synthesis of prostaglandin E_2 ethanolamide from anandamide by cyclooxygenase-2. *J Biol Chem*, **272**: 21181 - 21186.
Zhang Y, Chen K, Sloan SA, Bennett ML, Scholze AR, O'Keeffe S, Phatnani HP, Guarnieri P, Caneda C, Ruderisch N, Deng S, Liddelow SA, Zhang C, Daneman R, Maniatis T, Barres BA, Wu JQ (2014). An

RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. *J Neurosci*, **34**: 11929 - 11947.
Zhao S, Mugabo Y, Ballentine G, Attane C, Iglesias J, Poursharifi P, Zhang D, Nguyen TA, Erb H, Prentki R, Peyot ML, Joly E, Tobin S, Fulton S, Brown JM, Madiraju SR, Prentki M (2016). α/β-Hydrolase Domain 6 Deletion Induces Adipose Browning and Prevents Obesity and Type 2 Diabetes. *Cell Rep*, **14**: 2872 - 2888.
Zhao S, Poursharifi P, Mugabo Y, Levens EJ, Vivot K, Attane C, Iglesias J, Peyot ML, Joly E, Madiraju SR, Prentki M (2015). α/β-Hydrolase domain-6 and saturated long chain monoacylglycerol regulate insulin secretion promoted by both fuel and non-fuel stimuli. *Mol Metab*, **4**: 940 - 950.
Zhong P, Pan B, Gao XP, Blankman JL, Cravatt BF, Liu QS (2011). Genetic deletion of monoacylglycerol lipase alters endocannabinoid-mediated retrograde synaptic depression in the cerebellum. *J Physiol*, **589**: 4847 - 4855.
Zhou Y, Howell FV, Glebov OO, Albrecht D, Williams G, Doherty P (2016). Regulated endosomal trafficking of Diacylglycerol lipase alpha (DAGLalpha) generates distinct cellular pools; implications for endocannabinoid signaling. *Mol Cell Neurosci*, **76**: 76 - 86.

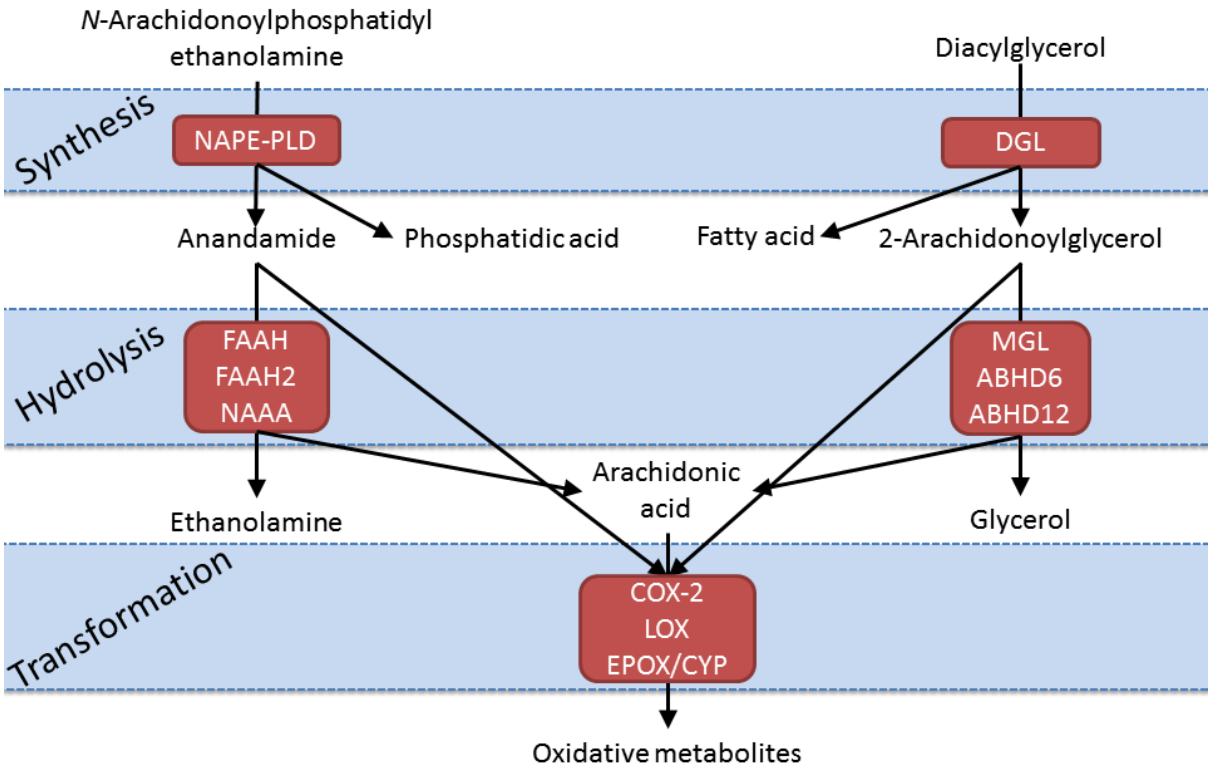
Table 1: Pharmacological tools used to investigate endocannabinoid turnover

Compound	**Comment**
Biochanin A	A reversible inhibitor with a modest selectivity for FAAH-2
DH376	A selective DAGL inhibitor
DO34	A selective DAGL inhibitor
JJKK048	A potent, selective MAGL inhibitor
JNJ-1661010	An irreversible inhibitor with a modest selectivity for FAAH-2
JZL184	A selective MAGL inhibitor, with reduced potency at the rat enzyme compared to human and mouse
KT185	An orally-active inhibitor of ABHD6
LEI015	An orally-active, reversible DAGL inhibitor
MAFP	A non-selective irreversible inhibitor of many eCB-metabolising enzymes, including FAAH, MAGL, ABHD6, ABHD12
OL-135	A reversible inhibitor with a modest selectivity for FAAH compared to FAAH-2
RHC80267	A DAGL inhibitor, which fails to inhibit ABHD12
Tetrahydrolipstatin	A DAGL inhibitor, which also inhibits ABHD12 and pancreatic lipase
URB597	An irreversible inhibitor with a modest selectivity for FAAH compared to FAAH-2
WWL123	An ABHD6 inhibitor
WWL70	An ABHD6 inhibitor

For further information and the appropriate citations, please refer to the text

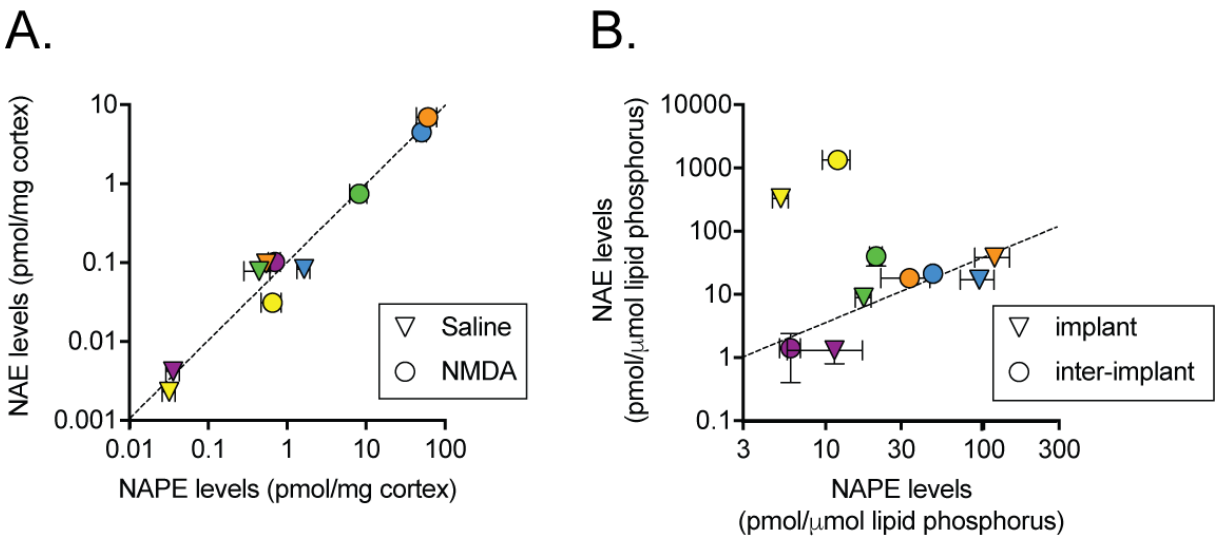
Legends to figures

Figure 1. Parallel and convergent pathways of endocannabinoid metabolism



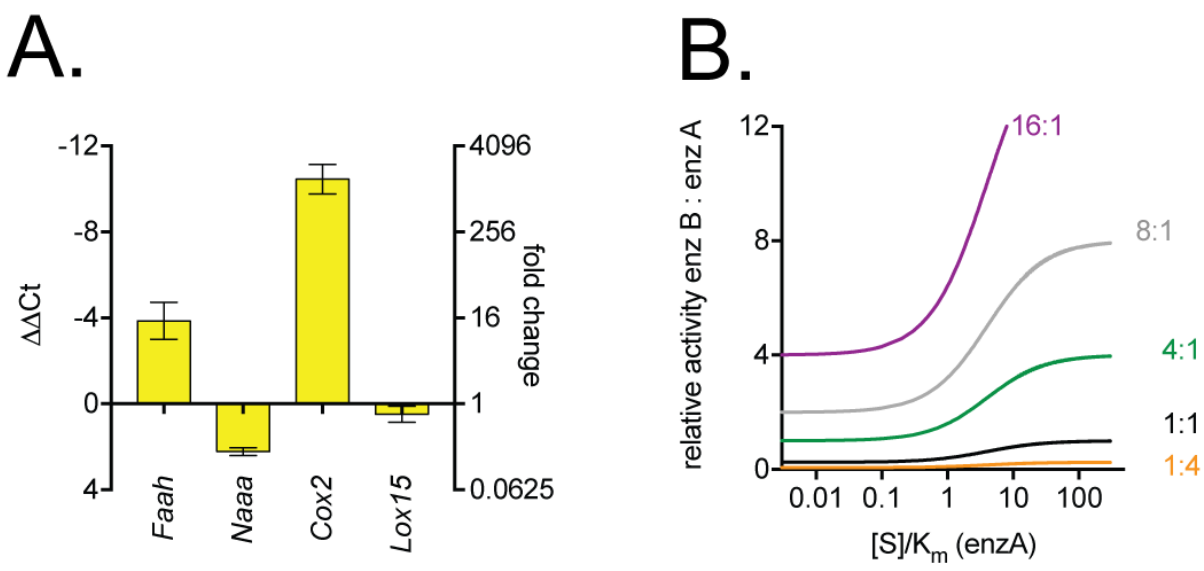
The graphic illustrates three phases of endocannabinoid turnover: synthesis, hydrolysis and transformation. For clarity, a number of alternative, apparently minor pathways of endocannabinoid metabolism are not shown.

Figure 2. Regulation of NAE synthesis.



Panels A and B. Levels of AEA (yellow), palmitoylethanolamide (PEA, blue), stearoylethanolamide (SEA, orange), oleoylethanolamide (OEA, green) and linoleoylethanolamide (purple) in relation to their corresponding NAPEs. In panel A, the values (means ± SEM, when not enclosed by the symbols, N= 3-6) are for cerebral cortices from rat pups 24 h after intrastriatal injection of either saline or 25 nmol of NMDA, and are drawn from the data of Hansen et al. (2001a and b). The regression line was calculated from the mean logged values and has a slope of 0.99 ± 0.06. In panel B, the values (means ± SD, N=3) are for uterine implantation and inter-implantation sites for female mice on pregnancy day 7, and are drawn from the data of Schmid et al. (1997). The regression line, calculated from the mean logged values for all NAEs except the two AEA values, has a slope of 1.04 ± 0.34. The highest mean value of AEA (1345 pmol / µmol lipid phosphorous) corresponds to 20 pmol / mg tissue.

Figure 3. Relative expression of the AEA catabolic enzymes: regulation and consequences.



Panel A. Changes in the mRNA expression of *Faah*, *Naaa*, *Cox2* and *Lox15* in RAW264.7 cells following 24 h incubation with lipopolysaccharide and interferon-γ. The data are shown as mean ± 95% bootstrapped percentile confidence limits of the difference in the ΔCt values between untreated and treated cells, n=7-16. A ΔΔCt of x corresponds to a 2^{-x} fold change, and this is shown on the right axis. The bootstrapped percentile confidence limits of the difference were calculated using 100000 iterations with the package Hmisc for the R computer programme. (drawn from data recalculated from the study of L. Gabrielsson, S. Gouveia-Figueira, M. Alhouayek & C.J. Fowler, submitted for publication). Panel B shows the relative activities for two enzymes A and B at different relative concentrations (shown as enz B : enz A in the figure) of the two enzymes, calculated assuming both enzymes have the same k_{cat}, while the K_m for enzyme A is four times lower than that for enzyme B. To our knowledge, such kinetic constants for FAAH and COX-2 have not been presented where the enzymes have been assayed under the same conditions (i.e. pH, presence or absence of detergent etc.). The family of curves demonstrate that the metabolic pathway of the substrate, i.e. via enzyme A or B, clearly depends on the ambient concentration and relative expression of the enzymes.